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*Drosophila melanogaster*  
DEGREE FOR WHICH THESIS WAS PRESENTED: Doctor of Philosophy  
YEAR THIS DEGREE GRANTED: 1973

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SEX-LINKED AUXOTROPHIC MUTATIONS

IN *DROSOPHILA MELANOGASTER*

by



Darrel R. Falk

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE  
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

FALL, 1973





THE UNIVERSITY OF ALBERTA  
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Sex-linked Auxotrophic Mutations in *Drosophila melanogaster* submitted by Darrel R. Falk in partial fulfilment of the requirements for the degree of Doctor of Philosophy.





## ABSTRACT

Thirty-two auxotrophic (or putative auxotrophic) mutations of *Drosophila melanogaster* have been isolated. The mutations result in low viability or slow development rate on a chemically defined medium but not on the more complete, yeast-sucrose medium. All mutants were tested for supplementation by RNA or ribonucleosides. On the basis of this test the mutations are of three types.

Eight mutants (at seven loci) do not respond to RNA and the nutritional requirement of each of these strains remains undefined.

Four of the 24 mutations which do respond to RNA are supplemented by a purine nucleoside. One of these (*ade 1-1<sup>sd</sup>*) is adenosine supplementable and maps to a position distinct from the other three. Of these other three, one (*gua 1-1<sup>ts</sup>*) responds to guanosine, and the others (*pur 1-1* and *pur 1-2*) are supplemented by either nucleoside. These mutations map in the same region and it has been suggested that *pur 1* and *gua 1* are distinct, but closely linked genes involved in purine biosynthesis.

Twenty of the mutations are supplemented by a pyrimidine nucleoside. All map close to *rudimentary* (*r*, 1-54.5), a site previously identified by wing abnormalities, and more recently established as a site of pyrimidine auxotrophy. About half of the newly isolated mutations have normal wings and yet, by a variety of criteria, do appear to be affecting the *rudimentary* locus. The data presented herein indicates that auxotrophy is a phenotype more



sensitive to reduced endogenous pyrimidine synthesis than previous morphological criteria. This sensitivity has permitted the isolation of a series of mutations with a spectrum of phenotypic abnormalities, which apparently reflects the degree to which pyrimidine biosynthesis is blocked.





## ACKNOWLEDGEMENTS

The work reported here is very much a cooperative effort and certainly would not have been possible without the assistance of a number of persons. In particular, I thank Don Morrison, Maureen Starko, Brendan Veilleux, Kin Wong and Barbara Yeung for their technical assistance in various stages of the work.

For making the lab not only a scientifically inspiring, but also a relaxing and enjoyable place to work, I acknowledge Mahmoud el Kouni, Fardos Naguib, and Patricia Romans.

For generating enthusiasm where I was too skeptical, skepticism where I was too bold, and perception where I was slow to perceive, I express sincere appreciation to my supervisor, David Nash. To study under him has been a privilege.

Finally, for typing this thesis and for her support, understanding and patience, which are a bigger part of this thesis than anyone will ever know, I thank my wife, Joyce.

Financial assistance was provided by National Research Council Grant A-3269 to Dr. David Nash and National Research Council post-graduate scholarships to myself.





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## INTRODUCTION

### 1. THE PROBLEM

The organization of the genome and the regulation of genetic activity has been the subject of very active research for many years. The explosive success of these studies over the past fifteen years has resulted in the elucidation of the basic biochemical and genetical mechanisms associated with gene expression in prokaryotes. However, during most of this time eukaryotes have remained in the background and, as a result, the universality of prokaryote findings is as yet unknown. There is a number of indications that genes may be organized and regulated quite differently in higher organisms. For example, their DNA usually contains some sequences which are repeated many times (Britten and Kohne, 1968). Such sequences are specific to higher organisms and it has been suggested (Britten and Davidson, 1969) that they play an important, but as yet undefined role in gene regulation. The association of higher organism DNA with histones represents a second distinction. Like some bacterial regulatory proteins, histones are capable of gene repression (Huang and Bonner, 1962). However, the limited variety of histones suggests that unlike their bacterial counterparts, they cannot of themselves dictate much specificity to gene repression (Fambrough et al, 1968). Moreover eukaryotes are distinguished from prokaryotes by the presence of a nuclear membrane. This allows for a processing of RNA and thus a



regulation of gene expression at the translational level to a degree not possible in the more open, less compartmentalized prokaryotic cells where transcription and translation appear to be coupled reactions (Morse et al, 1969). Multicellular organisms are further distinguished by tissue diversity and many tissues perform a specialized function throughout their whole life span. Therefore, such cells are often rather restricted in their metabolic capabilities. On the other hand, unicellular organisms must be capable of rapid alterations in metabolic activity if they are to survive in the wide variety of environmental conditions to which they are subjected. Thus, regulation of gene activity of these cells may be quite different than in multicellular organisms where flexibility is not required to the same degree.

All of these points suggest that even though tremendous insight has been gained regarding the nature and regulation of genes in prokaryotes, at least as great a challenge lies ahead. The challenge is to apply the knowledge and techniques which have accumulated from the very successful prokaryote studies to the detailed analysis of the gene and its regulation in higher eukaryotes. Studies on the replication and molecular composition of chromosomes, as well as transcription and the molecular biology of RNA are beginning to provide insight in this area. But the real success in elucidating gene organization and control in prokaryotes came about through a different approach. It was based on the assumption that the understanding of the *normal* role of a particular gene can be inferred from observation and analysis of the *abnormalities* resulting from mutation of that gene. In this way, the nature and role of particular genes has been elucidated





in a remarkably refined manner.

The success of this approach has often come through the analysis of mutants which are lethal under one set of nutritional conditions, but not another. Since the biochemical deficiency of such strains can be deduced from the nature of the nutritional alteration necessary to promote growth, these mutants are not usually difficult to characterize. Their usefulness in regulatory studies has depended upon:

- a. the identification of a number of genes affecting the *same* biochemical pathway, and
- b. the conditional lethality of mutations of these genes so that rapid genetic analysis is feasible.

In view of these advantages and their successful exploitation in prokaryotes, it would seem that the application of nutritional mutations to higher eukaryotes should have received enthusiastic consideration. However, the contrary has been the case and until recently even the question as to their existence had hardly been examined. There is a number of reasons for this. In bacteria most nutritional mutations involve a metabolic block in the *de novo* synthesis of a required end product. In multi-cellular animals however, the number of such *de novo* pathways is extremely reduced and furthermore, the possibility exists that *de novo* pathways which do exist, may not have been eliminated through selection because they are unconditionally required. Mutants in such genes would be lethal and not analyzable by this technique. A further problem is that the organization of the genome may be such that no nutritional mutants



could occur due to genetic redundancy, multiple pathways, or mitochondrial supplementation. Even if nutritional mutations could be induced, their isolation and analysis demands the use of a chemically defined medium so that the concentration of nutritional components can be regulated precisely or components eliminated completely if necessary. All of this work must be done under axenic conditions if the medium is to remain chemically defined and this is often a frightening task when dealing with large numbers of multi-cellular organisms. A further limitation is the necessity for an organism which can be easily manipulated genetically. Multi-cellular organisms are generally diploid and therefore extensive breeding is necessary for the isolation of strains homozygous for such mutations.

In light of these many obstacles, it is no surprise that work in this area has until very recently, been almost non-existent. But recent results of Vyse (1969) and Vyse and Nash (1969) suggest that many of these apprehensions may be unfounded. Their results suggest that *Drosophila melanogaster* may be suitable for nutritional mutation studies. This is probably the only multi-cellular animal which can presently be analyzed and manipulated at the level necessary for the isolation and thorough study of nutritional mutants. A chemically defined medium has been devised and it is possible to culture the organism axenically without great difficulty.

This thesis is addressed to the question of the degree to which such mutations exist in *D. melanogaster*. The results of this study indicate that there is probably a great number of genes susceptible to nutritional analysis in this organism. Furthermore, it



is now apparent that these mutations are not particularly difficult to isolate, and that abnormalities in at least some strains can readily be ascribed to particular biochemical pathways. Initial studies on several of these loci suggest that this type of analysis will be particularly fruitful in studies on the nature of gene organization and regulation in multi-cellular organisms.

## 2. NUTRITIONAL CHARACTERISTICS OF WILD TYPE *DROSOPHILA MELANOGASTER*

From the outset it is apparent that heritable nutritional abnormalities in *Drosophila* will present a different spectrum from those of bacteria and fungi. Animals lack a great many of the bio-synthetic capacities which are available for analysis in lower organisms. Many lower organisms synthesize all the organic components that they need from a simple sugar. On the other hand, *Drosophila*, like other animals is incapable of synthesizing a great many components and these must be nutritionally supplied. The determination of such nutritional requirements is important to nutritional mutation studies so that non-essential components can be excluded from the diet and later used as potential supplements of nutritional lethal mutations.

The nutritional requirements of *D. melanogaster* are well known. The first chemically defined culture medium was reported by Schultz, St. Lawrence and Newmeyer in 1946. This medium and a modified version (Hinton, Ellis and Noyes, 1951a) gave extremely slow larval development compared with the conventional, undefined media commonly





used for laboratory rearing of *Drosophila*. However, thorough analysis of nutrient optima (Sang, 1956) subsequently resulted in an improved chemically defined medium on which there is little difference in larval growth rate or survival compared with the same tester strain on a killed yeast medium.

The medium designed by Sang contains the milk protein, casein, as a source of amino acids, and hence is not strictly speaking a minimal medium since a number of amino acids are not essential. Previous media (Schultz et al, 1946; Hinton et al, 1951a) and a more recent medium devised by Geer (1965) contain a more restricted amino acid mixture, but Sang found a very marked improvement in growth rate by using casein. It has been suggested that this might implicate the existence of a stimulatory polypeptide (Schultz et al, 1946; Sang, 1956), but Dadd (1970) has suggested the possibility of imbalance in the amino acid mixtures. Alternatively, high concentrations of amino acids may adversely affect ingestion, either by inhibiting food intake, or by affecting osmotic relationships of the gut (Dadd, 1970).

Since insects cannot synthesize steroids *de novo*, cholesterol or one of several alternative steroids (Cooke and Sang, 1970) must be dietetically supplied. Steroids serve as a structural component of cells and tissues and also as precursors for essential metabolites and regulators such as ecdysone (Robbins et al, 1971).

Choline, which is an important constituent of lipids is another essential nutrient for which *Drosophila* has little or no synthetic ability. Choline is a component of lecithin and it is often



added in this form since lecithin, although not essential for growth, does have a stimulatory effect upon growth rate (Sang, 1956).

*Drosophila*, like other insects (review by Dadd, 1970), requires the B vitamins: thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid and biotin. Thiamine, which is necessary for larval survival, is converted intracellularly to thiamine pyrophosphate, a co-enzyme for certain carbohydrate metabolic reactions involving the transfer of an aldehyde group. Riboflavin is a precursor to the co-enzyme FAD, which is an essential component of the electron transport chain and is also important in the oxidation of certain amino acids and the purine, xanthine. Nicotinic acid, another required B vitamin, is necessary for the synthesis of NAD. And, subsequent to its conversion to pyridoxal phosphate, pyridoxine is important to the interconversion of amino acids.

Dependence upon particular vitamins is not always manifest similarly. Absence of any of the four vitamins discussed in the preceding paragraph results in lethality early in larval development, but this is not always the case. The omission of pantothenic acid, an acetyl CoA precursor, results in cessation of development but not in immediate death. Larvae can continue to live in its absence for up to 29 days (Hinton et al, 1951a) before death finally ensues. Dietary folic acid, on the other hand, is necessary for eclosion, but not for pupation. Since this vitamin is converted to tetrahydrofolate, an important methyl donor in purine, thymidylate and certain amino acid syntheses, this result is somewhat surprising. However, Sang (1956)



has demonstrated that larvae have a higher internal concentration of folic acid than the yeast upon which they feed and he has suggested that this may be indicative of some folic acid synthesis during the larval stage. Recent biochemical data on *Aedes aegypti* and *Drosophila melanogaster* suggest that this is indeed the case (Venters, 1971).

Biotin, a co-factor of some carboxylation reactions, increases the survival and growth rate of larvae but is not an absolute requirement for larval survival. Since a few individuals can complete development without its dietary inclusion, Sang suggests (1956) that this vitamin is probably present in trace amounts with some of the other ingredients. (For a detailed discussion of the B vitamins and their role in metabolism, see Robertson, 1966).

Several other non-essential components of Sang's (1956) medium stimulate the growth rate and increase the survival. One such component is a source of carbohydrate, fructose being the most satisfactory. The lack of requirement for carbohydrates is a rather striking result, especially when compared to the nutrition of most other species of insect larvae, which have rather high requirements (Dadd, 1970). Indeed, adult females of *D. melanogaster* have a very stringent carbohydrate requirement for the production of viable eggs, which further emphasises the uniqueness of the metabolism of developing *Drosophila* larvae in this respect.

Sang (1956) shows that a source of ribonucleotides is not required, although the presence of RNA does decrease larval development time by 3.5 days. In a later study (1957) he demonstrates that this increased growth rate cannot be completely attributed to any one





ribonucleoside or base, rather both a purine and pyrimidine source are required.

To summarize, Sang's medium gives a development rate and survival comparable to conventional undefined media. For this reason, even though it is not, strictly speaking, a minimal medium, it is the medium used as a restrictive medium in the present experiments. The required components (with Sang's constituents in brackets) are:

- a. a source of required amino acids (casein)
- b. a sterol source (cholesterol)
- c. a choline source (lecithin)
- d. most B vitamins

The non-essential components which have a significant effect upon survival and growth rate are:

- e. the B vitamin, biotin
- f. a carbohydrate source (fructose or sucrose)
- g. a nucleotide source (RNA)

For the specific reason that it is likely that many steps in nucleotide synthesis may yield auxotrophic mutants, component (g) was not normally included in the restrictive medium.

The development of a defined medium on which growth rate and survival are essentially equivalent to that of the conventional undefined media is a major accomplishment. However, Geer's (1963) analysis of viability and development rate for several strains on Sang's medium suggests that there is variation in what is an optimal



medium for different strains of flies. "Canton-S", for example, survives poorly unless RNA or, alternatively a purine and pyrimidine are present as additives. The demonstration that changes in the amino acid balance or the use of hydrolyzed casein partially obviate the need for nucleotide supplementation suggests that this strain is not necessarily deficient in nucleotide production. Geer (1963) suggests that "Canton-S" is subject to a developmental stress on Sang's RNA-free medium which is remediable by a variety of nutritional manipulations. This particular "developmental stress", like interstrain variation in general, might be explained by nutrient imbalance. The sensitivity of larvae to nutrient balance is illustrated by the demonstration that a change in the protein concentration shifts the optimal concentration of a number of vitamins (Sang 1959, 1962). For example, folic acid, a precursor to tetrahydrofolate, which is a co-factor in the methylation of glycine, is required in a higher concentration in a high protein medium. The increased folic acid requirement may be accounted for solely by increased glycine concentration in the high protein medium, since the addition of glycine to the low protein medium simulates the high protein effect. Most other shifts in optimal vitamin concentrations can, likewise, be demonstrated to result from specific interactions (Sang, 1962).

From these studies, it becomes apparent that the quantitative nutritional requirements are not rigid. Geer (1963) has shown them to be very sensitive to genotypic variations. Sang (1959, 1962) has shown them to be extremely sensitive to variation in nutrient balance. And lastly, it seems likely (although no evidence exists for *Drosophila*),



that such requirements are also sensitive to environmental variations such as temperature. The only organism for which I am aware of studies on the effect of temperature variation, the protozoan, *Ochromonas malhamensis*, has much different nutritional requirements when reared at high temperatures (Hutner et al, 1957).

Even though the quantitative aspects of *Drosophila* nutrition fluctuate, the basic requirements are probably quite uniform and thus serve as a foundation for the study of nutritional abnormalities.

### 3. NUTRITIONAL MUTANTS IN MULTICELLULAR ANIMALS

Mutants which respond abnormally to a particular nutritional environment have received very little attention in higher organisms. Some mutant strains have been isolated in higher plants (Carlson, 1969; Li et al, 1967), but most of the results have come from several vertebrate cell lines or in the case of complete organisms, *D. melanogaster*. The remainder of this discussion will concentrate on nutritional mutants as they have been studied in animals.

#### a. Mutants with Nutritionally Modifiable Morphological Defects

One approach to defining the biochemical deficiency of a mutation is to check pre-existing morphologically abnormal strains for sensitivity to alteration of the nutritional environment. The essence of this approach is that the nature of the nutritional modification to which the mutant responds might provide insight into the abnormality





at the metabolic level. To the present, this method has not been particularly illuminating.

The mutant, *antennaeless* of *D. melanogaster* is a nutritionally responsive strain (Gordon and Sang, 1941). The mutant effect can be enhanced by addition of peptone to a sterilized brewer's yeast medium, or alternatively it can be suppressed by riboflavin (Gordon and Sang, 1941) or glucose (Begg and Sang, 1945). Gordon (1959) has proposed that these data indicate a defect in amino acid metabolism. He suggests that this defect is remediable by a higher level of oxidation of amino acids (the riboflavin effect) or by a decrease in the utilization of amino acids for energy-producing oxidations (the glucose effect). However, the critical biochemical tests have never been performed.

Several melanotic tumour producing strains of *D. melanogaster* are also sensitive to nutritional environment. The tumours result from an aggregation of certain blood cells and are generally quite infrequent in these mutant strains on Sang's defined medium. However, when larvae are cultured on media with reduced concentrations of adenosine, cholesterol or biotin (Sang and Burnet, 1963) or on a medium with a high level of tryptophan (Plainer and Glass, 1955), the tumour frequency is increased. Sang and Burnet (1963) suggest that the gene-controlled defect is near the limit of a developmental buffering, and under conditions of stress such as those described above the buffering system can no longer maintain that developmental pathway which leads to the normal phenotype.



The eye-reducing mutant, *Bar*, is nutritionally modifiable. A number of amides, cytosine, uracil and adenine increase the number of eye facets, in some cases to a level approaching that of wild type (Kaji 1954, 1955; De Marinis 1966 a,b). Fristrom (1969) has shown that the *Bar* phenotype results from cellular degeneration. Acetamide reduces such degeneration drastically, whereas cytosine delays the degeneration beyond the stage where a mutant effect on facet number is produced (Fristrom, 1969).

The study of the eye colour mutant, *vermilion* is one case when nutritional studies have been successful in elucidating the biochemical basis of a morphological abnormality. Dietary kynurenine changes the bright red eye colour of *vermilion* mutants to the normal dull red (Tatum and Haagen-Smith, 1941). This finding led to the proposition that mutants are unable to synthesize kynurenine from tryptophan, a theory subsequently verified through biochemical studies (Baglioni, 1960).

The limitations of this approach to the metabolic delineation of mutant effects probably has several explanations. Certainly, the fact that only a few mutations have been nutritionally analyzed is a factor, but probably more important is that morphological mutants have been isolated on the conventional undefined media, where many nutrients are present in abundant supply. It seems likely that if this method is to succeed, morphological mutations will need to be isolated which are abnormal solely on a minimal medium.



b. Mutants Which are Viable on a Normally Toxic Medium

Determining the chemical specificity of a mutant effect requires that the potential explanations be limited in number and easily narrowed down. In higher eukaryotes, the technique which has best fulfilled these requirements is the analysis of mutations which confer resistance to normally toxic chemical analogues. Since the cause of analogue susceptibility is generally quite specific, the mechanism of resistance to this toxicity is also specific and thus readily characterizable. Resistance mutations are usually of three types:

- i. Those which prevent the entry of the analogue into the cell.
- ii. Those which prevent the accumulation of the active, inhibitory form of the analogue.
- iii. Those which alter the specificity of the sensitive enzyme in such a way that it is not inhibited by the analogue.

Mutations of all three types have been found in studies with tissue culture and tumour lines. Breslow and Goldsby (1969) isolated several lines of Chinese hamster cells which are resistant to a normally toxic radioactive level of  $H^3$  thymidine. Such strains are deficient in the uptake of thymidine from the medium. A number of cell culture strains have been isolated which are resistant due to an inability to accumulate the inhibitory form of the analogue. For





example, a low activity of the enzyme, hypoxanthine-guanine phosphoribosyl transferase (which converts base to nucleotide) is commonly associated with strains resistant to 8-azahypoxanthine or 8-azaguanine (Szybalski and Szybalska, 1962; Chu et al, 1969). Strains resistant to the glucose analogue, 2-deoxyglucose, have an elevated activity of alkaline phosphatase (Morrow and DeCarli, 1967). 2-deoxyglucose is believed to exert its effect after phosphorylation (Barban and Schulze, 1961). The increased alkaline phosphatase is supposed to decrease this toxic form. Other examples of this type include the absence of thymidine kinase activity in a bromodeoxyuridine (BUdR) - resistant strain of mouse L cells (Kit et al, 1963) and low uridine kinase in Erlich Ascites tumour cells resistant, to fluorouracil (FU) (Reichard et al, 1962).

A mutation of the third type referred to above has been demonstrated for the enzyme, thymidylate synthetase. This enzyme converts deoxyuridine monophosphate (dUMP) into thymidine monophosphate (TMP). Fluorodeoxyuridine monophosphate (FdUMP) inhibits this reaction (Cohen et al, 1958). An FU-resistant strain of Erlich Ascites tumour cells is resistant through an alteration of the thymidylate synthetase molecule, such that it no longer is inhibited by FdUMP (Heidelberger et al, 1960).

Although the biochemical characterization of these mutants has been very successful, studies with tissue culture lack the tools for the genetic refinement possible with *Drosophila*. Several experiments of this type have recently been reported for *Drosophila*. One experiment applies the fact that pentenol is selectively toxic to



flies exhibiting ADH activity. Mutants deficient for this enzyme are able to live, presumably because they are unable to convert pentenol to the poisonous ketone form (Sofer and Hatcoff, 1972). El Kouni (1972) has demonstrated that the toxic effect of fluorodeoxyuridine (FUdR) on Sang's medium is overcome by thymidine. This suggests that, as in tissue culture, the effect of this analogue is on the enzyme, thymidylate synthetase. Recently an FUdR-resistant strain has been isolated and resistance apparently results from an autosomal dominant mutation (M. el Kouni, personal communication). Sherald and Wright (1972) have reported the isolation of three strains resistant to the toxic 3,4-dihydroxyphenylalanine (dopa) analogue,  $\alpha$ -methyl dopa. At least two of the strains have an elevated activity of dopa decarboxylase and it has been suggested that it is this elevated activity which allows the flies to survive in the presence of the inhibitor.

#### c. Mutants Which are Lethal on a Normally Permissive Medium

Nutritionally remediable lethal mutations have received very little attention in higher eukaryotes. Theoretically, as with analogue-resistance mutations, chemical specificity should not be difficult to determine, since the nature of the nutritional alteration which promotes growth should disclose the deficient pathway. Because a number of different mutant enzymes affecting the same pathway can be identified, this approach provides a broader spectrum of mutations than the chemical analogue technique. In bacteria this has proven to be a



major advantage for the investigation of co-ordinate regulation of functionally related enzymes. Recent results indicate that it may be similarly applied to higher organisms.

Kao and Puck (1968) have applied a rather ingenious approach to the isolation of auxotrophs in Chinese hamster cells. Cells treated with a mutagen are grown in the presence of BUdR. Those cells which are metabolically active incorporate BUdR into their DNA and are subsequently killed upon exposure to near-visible light. Nutritional mutants, on the other hand, are metabolically inactive and thus are not killed because of a lack of BUdR incorporation. Such mutants begin to grow when shifted to a BUdR-free, complete medium. By this technique an inositol auxotrophic strain, several glycine-requiring strains and a number of strains with a multiple requirement for glycine, hypoxanthine, and thymidine were isolated. The latter strains were thought to be mutant in folic acid reductase since the product of this enzyme (tetrahydrofolate) is necessary for the production of all three of the required supplements or their derivatives.

A similar technique has been used for the isolation of glutamine auxotrophs in Chinese hamster cells (Chu and Malling, 1968; Chu et al, 1969). In these experiments aminopterin was used to create a lethal thymine deficiency in actively growing cells on a medium containing no glutamine. When such cultures were transferred to a medium free of aminopterin, but containing glutamine, clones were produced, some of which proved glutamine dependent.

In *Drosophila*, several attempts at the isolation of





nutritionally supplementable lethal mutations have been made. One such attempt resulted in the demonstration that a pre-existing strain carrying the inversion, In (2LR) 40d was lethal in the absence of adenine (Hinton et al, 1951b). Later studies indicate however, that this strain is probably not deficient in the production of adenine since replacing free amino acids of the early medium by casein as the amino acid source, also promotes growth (Ellis, 1959). Nutritional requirements of several other pre-existing strains were similarly difficult to analyze, since they were not the result of a single genetic defect (Hinton, 1959; Ellis, 1959).

Unfortunately these negative results discouraged further analysis and it was not until recently that specific metabolic deficiencies have been demonstrated to result in nutritional conditional lethality. The first to demonstrate such an effect were Grell, Jacobsen and Murphy (1968), who showed that adults with no alcohol dehydrogenase are lethal on a medium containing a normally tolerable, 15% ethanol. Later, several strains (*rosy*, *maroonlike*) with low activity of the purine degradative enzyme, xanthine dehydrogenase were shown to be lethal as larvae, on a high purine medium (Finnerty, Baillie, and Chovnick, 1970). In another example, the pyrimidine dependency of the pre-existing wing mutant, *rudimentary* (Norby, 1970) is beginning to lead to the understanding of the normal metabolic role of this locus (Norby, 1973). Generally mutants mapping throughout one half of this locus are deficient for the second enzyme of the pyrimidine biosynthetic pathway, aspartate transcarbamylase, whereas





those in the other half are not (Norby, personal communication). Activity of the first pyrimidine biosynthetic enzyme, carbamyl phosphate synthetase has not yet been checked, but in yeast (Lacroute, 1968) and *Neurospora* (see Davis, 1967), the first two enzymes are closely linked. Norby (1973) suggests that in *Drosophila*, *rudimentary* mutants with normal aspartate transcarbamylase will prove to be deficient in the first enzyme, carbamyl phosphate synthetase, but this is a question currently under investigation.

It is now obvious that nutritional conditional lethal mutations exist in *Drosophila* and that they probably will prove extremely useful in analyzing the normal metabolic role of particular genes in detail. However, the work discussed above is the result of screening only a small number of pre-existing strains for nutritional abnormalities. If this approach to metabolic analysis of gene action is to reach its potential, mutagenesis, followed by screening for nutritional lethals will be a necessity. Vyse and Nash (1969) demonstrated the feasibility of this approach. They report that after chemical mutagenesis, three out of 377 X chromosomes carried a mutation resulting in a supplementable lethality on Sang's medium without RNA.

In the present investigation, over 5500 mutagenized X chromosomes have been screened for one class of nutritional conditional lethal mutations, auxotrophs. The subsequent analysis of the 32 mutants recovered, reveals a number of properties of probable interest to the understanding of gene action at the metabolic level.



## MATERIALS AND METHODS

### 1. STOCKS

All genetic manipulations were carried out with an inbred stock, or with stocks which had been repeatedly backcrossed to it. This precaution was taken to reduce the probability that genetic modifiers might accumulate and interfere with the expression of a mutation. The parental strain used was an Oregon derivative from Amherst College (see *Drosophila* Information Service, 1968, stock 1). Table 1 provides a summary of the various stocks which were used.

### 2. MAINTENANCE OF AXENIC CULTURES

Isolation and characterization of nutritional mutants was carried out on media free from microbial contaminants. In most previous nutritional studies, stocks have been maintained under non-axenic conditions and newly sterilized for each required test. Because of the scale of the present experiments, repeated sterilization of every strain for every test has been impractical. Therefore, techniques were devised for the long term maintenance of axenic cultures.

Strains are initially sterilized in the following manner. Eggs laid over a 12 hour period are scraped from a 1.5% agar medium and placed for 20 minutes in Kimax<sup>R</sup> culture tubes containing a freshly



TABLE 1 LIST OF *DROSOPHILA MELANOGASTER* STOCKS USED

STOCK NO. AND DESIGNATION	SOURCE	COMMENTS <sup>a</sup>	MAP LOCATIONS <sup>a</sup>
1. Amherst Oregon R	Amherst College Amherst, Mass.		
2. C(1)RM, $\gamma$ sc su( $w^a$ ) $w^a$ bb● $\gamma$ sc $\frac{4L}{8R}$ sc	Cal Tech.	a. Maintained as $\overline{XXY}$ with males of stock 1 b. Backcrossed into stock 1: 6 generations	
3. *In(1)FM7b, $\gamma$ $\frac{31d}{\#}$ $w^a$ 1z <sup>S</sup> B FNC4	* Cal. Tech. # Newly isolated in males of stock 2	* An effective balancer of the X chromosome ts lethal and female sterile a. Backcrossed to stock 1: 5 generations	
4. pn*v*m*wy*f#	* Newly isolated in males of stock 2 # from ycvvf of Cal. Tech.	pn - brownish purple eyes v - bright red eyes m - short wings wy - curled wings f - forked bristles backcrossed to stock 1: 5 generations	1;0.8 1;33.0 1;36.1 1;41.9 1;56.7

continued on next page





TABLE 1 - CONTINUED

LIST OF *DROSOPHILA MELANOGASTER* STOCKS USED

STOCK NO. AND DESIGNATION	SOURCE	COMMENTS <sup>a</sup>	MAP LOCATIONS <sup>a</sup>
5. $\frac{Y\ sc\ v\ f.Dp(1,1)sc^{vi}}{B^SY}$	J. Merriam, U.C.L.A.	Y - yellow bristles sc - bristles missing a. X chromosome carries y+ duplication on short arm b. Y chromosome carries a B <sup>S</sup> -bearing proximal segment of the X	1;0.0 1;0.0
6. Females: stock 2 Males: FNC4 (stock 3)		a. Virginator stock: only $\overline{XXY}$ females survive at 29°	

<sup>a</sup> Phenotype and map location of specific mutations are described only for markers used in mapping experiments. For more detailed description, see Lindsley and Grell (1968).



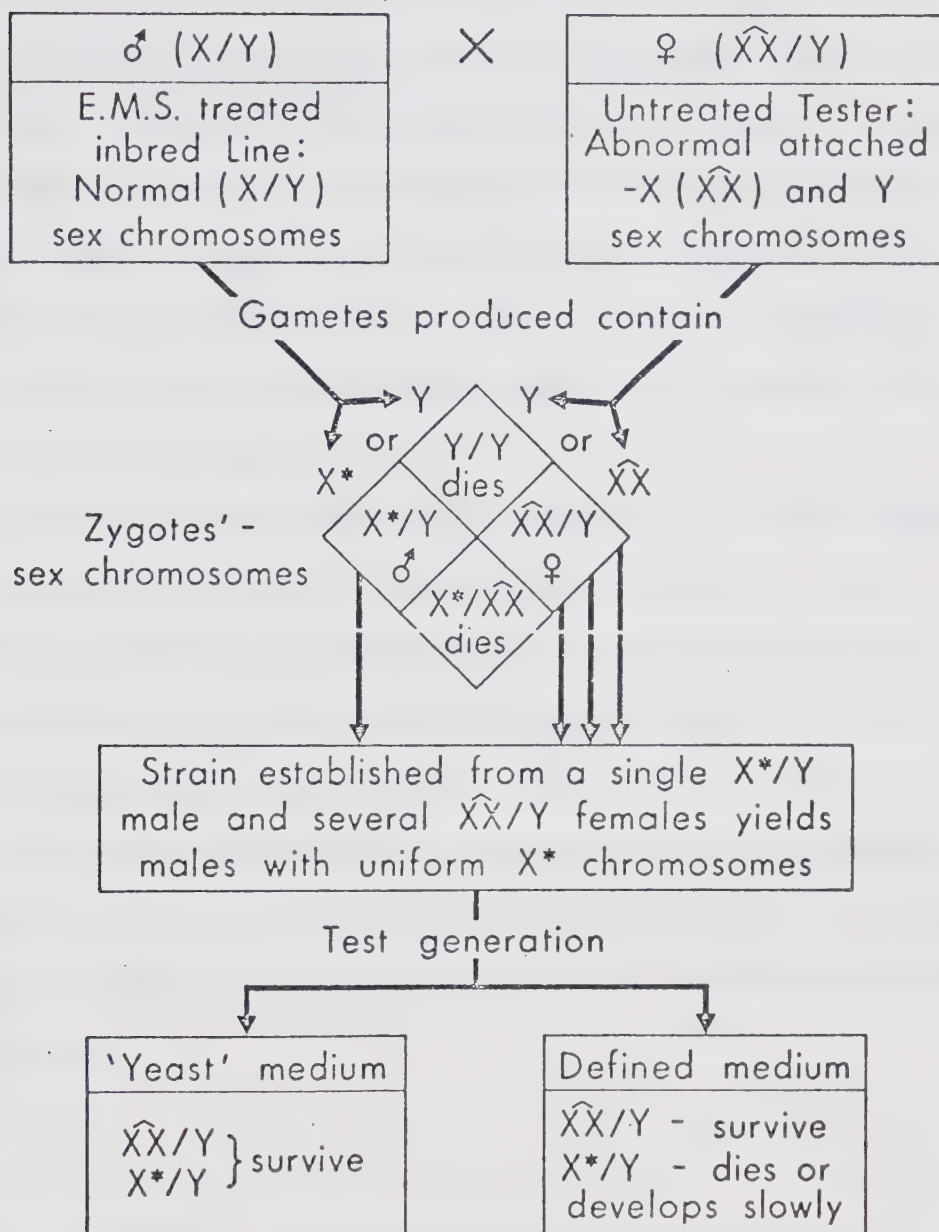
TABLE 2  
MEDIA USED IN SCREENING AND TESTING  
NUTRITIONAL CONDITIONAL MUTANTS

a. Defined medium (modified slightly from Bryant and Sang, 1969)			
Agar (Oxoid No. 3)	3.00 g.	Biotin	0.16 mg.
Casein (Vitamin Free)	5.50 g.	Folic acid	.3 mg.
Sucrose	750 mg.	NaHCO <sub>3</sub> (anhydrous)	140 mg.
Cholesterol	30 mg.	KH <sub>2</sub> PO <sub>4</sub> (anhydrous)	183 mg.
Lecithin	400 mg.	K <sub>2</sub> HPO <sub>4</sub> (anhydrous)	189 mg.
Thiamine	.2 mg.	MgSO <sub>4</sub> (anhydrous)	62 mg.
Riboflavin	.1 mg.	Streptomycin	17 mg.
Nicotinic acid	1.2 mg.	Penicillin*	25,000 I.U.
Ca pantothenate	1.6 mg.	Water	to 100 ml.
Pyridoxine	.25 mg.	RNA (when added)	400 mg.
b. Dead yeast-sucrose medium (modified from Nash and Bell, 1968)			
Brewers yeast	12.5 g.	Penicillin*	25,000 I.U.
Sucrose	10.0 g.	Propionic acid*	1.0 ml.
Granulated Agar	2.0 g.	Water	90 ml.
Streptomycin	25 mg.		
c. Microbial Culture Medium			
Agar	27.7 g.	Casein	5.0 g.
Yeast extract	5.0 g.	Water	to 1000 ml.

\* added after autoclaving



FIGURE 1      PROTOCOL FOR THE PRODUCTION AND ISOLATION OF  
SEX-LINKED AUXOTROPHS



X\* indicates a chromosome to be tested for  
nutritional mutations



filtered solution of 3% calcium hypochlorite which dechloronates and sterilizes the embryos. The suspension of embryos is filtered and the embryos are given three successive rinses with sterile Ringer solution. They are then scraped from the filter paper and placed in culture vials. Routine microbiological sterile technique is followed. Up to 12 different stocks can be sterilized simultaneously in about two hours. After at least five days, microbial contamination is checked by plating a small amount of medium from newly sterilized cultures on a microbial culture medium (Table 2c). Generally 80 - 90% of the cultures are axenic.

Initially stocks were maintained on antibiotic-free medium, but due to a high frequency of fungal contamination, this became impractical. Antibiotics (see Table 2b) are now added routinely. Axenic cultures are best maintained in vials, because they can be effectively capped with Kaputs (Bellco Glass Inc.). Cultures are periodically checked for microbial contaminants by plating samples of media, but this is generally unnecessary since infected cultures can be recognized visually. Normally, contamination occurs in less than 1% of cultures.

### 3. MUTANT SELECTION

The protocol for the isolation of nutritional mutants is shown in Figure 1. Since the technical, rather than genetic, aspects of this screen represent the results of several years of trials and





modifications, the method is described in detail below, on a generation basis. Following this procedure, it is possible for one person to screen approximately 1000 X chromosomes per month.

a. Mutagenic Treatment and the First Generation Cross

Adult males (less than 48 hours old) are fed the mutagen, ethyl methane-sulfonate (EMS) as described by Lewis and Bacher (1968). The standard concentration (25mM) of EMS essentially sterilizes Amherst males, therefore several lower concentrations have been used (see Table 3). The concentrations used yield, in this stock, 30 - 50% sex-linked recessive lethal mutants in a Muller-5 test (Nash, personal communication).

Mutagenized males were mated to virgin  $\overline{XX}/Y$  females which were collected from stock 6, using the virgination technique originally devised by Wright (1968). Since in an  $\overline{XX}/Y$  stock, the X chromosome of the male is inherited patroclinously (Figure 1), an X-linked temperature sensitive mutation can be passed exclusively through males. When such cultures are kept at 29<sup>0</sup>, only females survive and will thus be virgin.

The first generation was grown in vials containing sterile yeast-sucrose medium (Table 2b). Generally each culture contained five mutagenized males and fifteen  $\overline{XX}/Y$  females. Parents were discarded after seven days. Cultures were maintained at either 20<sup>0</sup> (screens 5 and 6, Table 3) or 25<sup>0</sup> (screens 1-4, Table 3).



b. The Second Generation

To reduce handling and sorting of flies,  $F_1$  cultures were checked twice per day for the emergence of males. In this way, most cultures had no more than one male and they could simply be transferred with females from the same vial to new yeast-sucrose vials. Because of the induction of sex-linked recessive lethals in  $F_1$  males, there were generally several females for every surviving  $F_1$  male. In cases where more than one male was present, they were separated (without etherizing or direct handling) and each male was given several females from the same tube. After one week of checking vials twice per day in this manner, parental cultures were discarded. Care was taken to keep record of those  $F_1$  males coming from the same parental culture so as to detect brothers carrying identical mutations, due to a pre-meiotic mutational event. (No such mutations were detected).

c. The Third Generation

About five days after the initial emergence of  $F_2$  adults, 15 - 20 flies were transferred (without direct handling) to vials containing Sang's defined medium (Table 2a) without RNA. These flies were allowed to oviposit for 24 - 48 hours after which they were discarded.

The yeast-sucrose cultures from the previous generation were maintained for a second generation without retransferring. The composition of the medium is critical to the elimination of this retransfer step. A medium with lower agar and yeast concentrations



frequently produces only one generation of flies.

Several days after the beginning of emergence, the defined medium cultures were scored for the absence of males. The adults from the corresponding complete medium culture of a prospective mutant line were then rechecked for nutritional conditional lethality or a nutritional conditional developmental delay of at least four days.

In one screen (screen 1, Table 3) strains which had a supplementable, semi-lethality or one - two day developmental delay were saved and rechecked. Such strains were subsequently checked at 29°C for temperature sensitive auxotrophy.

d. Temperature Sensitive Screen

In some of the later experiments, a more specific scheme for the isolation of temperature sensitive auxotrophs was designed. In such experiments (screens 4, 5 and 6, Table 3),  $F_2$  females were allowed to oviposit on defined medium for 48 hours at 25°C (screen 4) or 20°C (screens 5 and 6). Cultures were left at this temperature for an additional 24 hours to allow time for most embryos to hatch and then they were transferred to 29°C. As larvae began to pupate, the cultures were returned to the original temperature. The rationale behind such a screen is two-fold:

- i. To increase the spectrum of mutations through the detection of *ts* auxotrophs.





- ii. To isolate nutritional mutations in genes which are otherwise not analyzable in this manner, due to an absolute requirement for gene activity in the unsupplementable embryo and/or pupal stages. *Ts* mutations of such genes might be detectable if larvae are kept at 29° but the unsupplementable stages (embryo and pupae) are kept at permissive temperatures.

#### 4. CHARACTERIZATION OF MUTANTS

##### a. Confirmation of Mutant Effects

Mutant X chromosomes are maintained patroclinously in stocks with  $\overline{XX}/Y$  females. In testing such strains, approximately 10 - 15 flies from such stocks were allowed to oviposit on vials containing the defined medium for approximately 24 hours. (In some tests where synchronization of a culture is not important, they were allowed to oviposit for about 48 hours). Such cultures have an internal control since the survival of female offspring, but not males, or the delayed eclosion of males relative to females is indicative of an X-linked mutation. To confirm supplementability of such effects, identical tests were performed on yeast-sucrose medium.

A mutant effect was generally retested on at least three different batches of defined medium and a total of at least 20 single



vial cultures.

b. Supplementation Tests

Soon after the confirmation of a mutant effect, strains were tested for supplementation with RNA (0.4%) or a mixture of the four ribonucleosides, adenosine, guanosine, cytidine, and uridine (0.1%, each). Those mutants which did not respond have not been further tested with respect to supplementation and are referred to as putative auxotrophs. Those strains which do respond were tested with individual nucleosides (0.1%) and were categorized on the basis of this test as purine or pyrimidine auxotrophs. Tests were performed in  $\overline{XX}/Y$  cultures in the manner described above (10 - 15 adults per vial, for 24 - 48 hours). Experimental results generally represent the sum of at least 10 single vial cultures from at least two different batches of medium.

c. Mutant Nomenclature

The loci of putative auxotrophs are designated *yea* 1, *yea* 2, etc., on the basis of their yeast-sucrose supplementation. A hyphenated allele number follows this locus designation and it is followed by the superscript "ts" or "sd" in the case of temperature sensitive or slow developing auxotrophs respectively, e.g. *yea* 2-2<sup>sd</sup>.

Other mutations are similarly identified by their supplement, i.e. *pur*, *gua*, *ade* or *pyr*.

In some cases an auxotrophic mutation is associated with



a previously identified morphological locus. In such a case the mutant is given the symbol ascribed to it in Lindsley and Grell (1968), together with a superscript with its auxotrophic designation, e.g.  $r^{\text{pyr 1-1}}$ .

#### d. Mutant Localization

Mutations were localized using *pnv m wy f* (stock 4, Table 1). Mutant males were crossed to virgin *pnv m wy f* homozygous females on yeast-sucrose medium. The  $F_1$  offspring of this cross were allowed to mature for several days and then 10 - 15 adults per tube were placed on individual vials containing unsupplemented defined medium. Parents were transferred to fresh vials at 24 - 48 hour intervals. The genotype of the surviving male progeny were scored and from this data the approximate map position was determined (see Appendix, for details).

Several of the mutants were localized between *forked* and the centromere. The map position of these mutants was rechecked in the same manner as above, but using stock 5. The advantage of this stock for this purpose, together with the methodology of mutant localization, is described in the Appendix.

#### e. Complementation Tests

Mutations which had similar map positions were tested for complementation. The crosses which were used for this purpose are described together with the results in the appropriate tables.



f. Analysis of Pleiotropic Effects

i. All mutants were made homozygous and tested for fertility of homozygous females by the following series of crosses (\* indicates the mutation-bearing chromosome):

$$\begin{array}{rclcl}
 \text{P} & \frac{\text{FM 7b}}{\text{FNC 4}} & (\text{stock 3}) & \text{X} & \frac{*}{=} \\
 \\
 \text{F}_1 & \frac{\text{FM 7b}}{*} & & \text{X} & \frac{*}{=} \\
 \\
 \text{F}_2 & \frac{*}{*} & & \text{X} & \frac{*}{=}
 \end{array}$$

ii. Mutants were tested for viability on the complete medium at 29° and at 18°. This was performed by placing 10 - 15 adults of the  $\overline{\text{XX}}/\text{Y}$  culture of each mutant on the yeast-sucrose cultures and scoring male offspring relative to their  $\overline{\text{XX}}/\text{Y}$  sisters.

iii. Dominance - Most mutations were tested for dominant effects in conjunction with the *pn v m wy f* mapping experiments in the following manner:

$$\begin{array}{rclcl}
 \text{P} & \frac{pn \ v \ m \ wy \ f}{pn \ v \ m \ wy \ f} & & \text{X} & \frac{*}{=} \\
 \\
 \text{F}_1 & \frac{pn \ v \ m \ wy \ f}{*} & & \text{X} & \frac{pn \ v \ m \ wy \ f}{=}
 \end{array}$$





F<sub>2</sub>     The number of *pn v m wy f* females relative to + + + + + were scored. A reduction of + + + + + females is indicative of dominance.

iv. Wing Abnormality of Pyrimidine Auxotrophs - The degree of the *rudimentary* wing expression was quantified in the manner described by Green (1963). Ten females and ten males homozygous for the pyrimidine auxotrophy are scored as either 1, 2, 3, or 4 depending on the extremity of the mutant wings. This is shown in Figure 2.

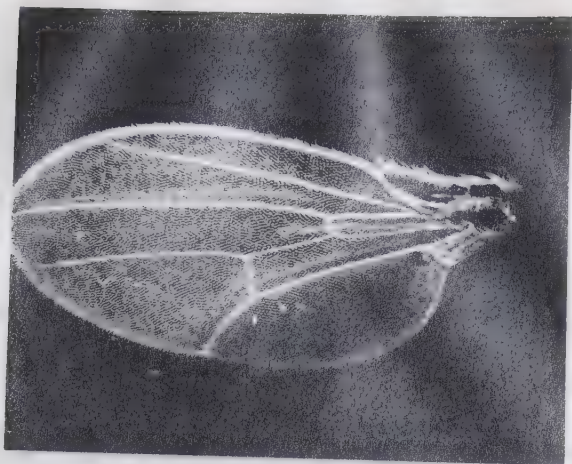




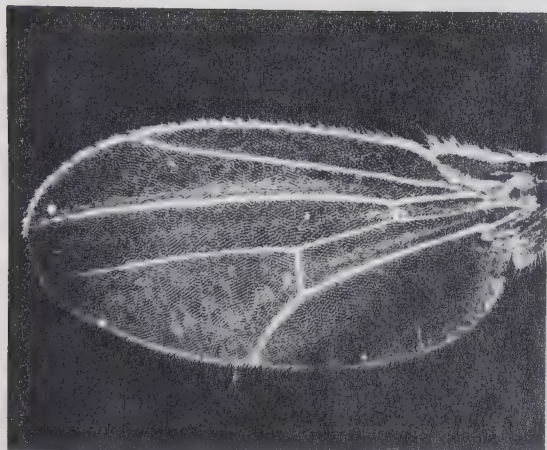
FIGURE 2

Categorization of wing phenotype of mutations at the *rudimentary* locus. (a) Amherst male; Score: 4. (b)  $(r)^{\text{pyr } 1-4}$  male; Score: 4. (c)  $r^{\text{pyr } 1-12}$  male; Score: 3. (d)  $r^{\text{pyr } 1-15}$  male; Score: 2. (e)  $r^{\text{pyr } 1-19}$ ; Score: 1. The method of scoring was derived from Green (1963). The rationale for the parentheses i.e. (r), in (b) above, is described at a later point (see p. 69).

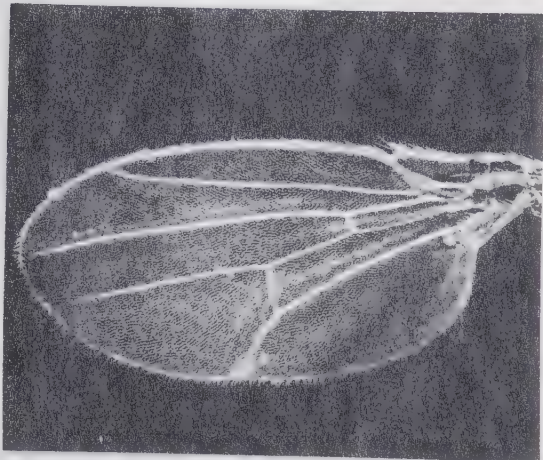
a.



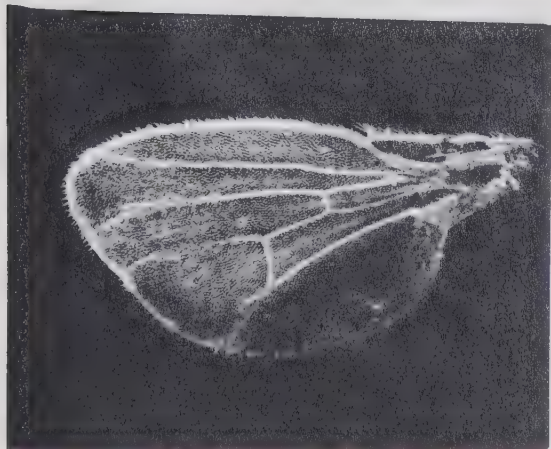
b.



c.



d.



e.







## RESULTS

### 1. THE SEARCH FOR MUTANTS

In the course of the mutant search, two basically different selection procedures have been utilized. The first is a constant temperature (25°) screen (screens 1 - 3, Table 3). The second includes a temperature shift (screens 4 - 6, Table 3) designed to recover larval temperature sensitive mutations.

Six of the 1694 X chromosomes (0.35%) analyzed in the constant temperature screens carried nutritional mutations complying with the established criteria (less than 5% viability or a three day developmental delay). In the temperature shift screens, 3961 X chromosomes were analyzed, of which 24 (0.60%) carried nutritional mutations. Eight of these (0.20%) showed temperature sensitivity<sup>1</sup>, whilst the remaining 16 (0.40%) showed characteristics which would have been evident at 25°C.

Screen 1 was less stringent in nature than the other five. In addition to the three mutations listed in Table 3, eight other strains were selected which have less extreme nutritionally supplementable defects. Two of these become extreme upon culture at

---

<sup>1</sup> A temperature sensitive auxotroph is hereby defined as one which does not comply with the mutant criteria (5% viability or, greater than three day developmental delay) at 25°, but does at 29°.



TABLE 3

SUMMARY OF THE NUMBERS OF MUTANTS ISOLATED  
IN DIFFERENT MUTAGENESIS EXPERIMENTS

SCREEN NO.	TEMPERATURE (°C) <sup>a</sup>	EMS CONC. (mM)	NO. OF X CHROMOSOMES TESTED	NO. OF MUTANTS <sup>b</sup> ISOLATED
1.	25	6.3	986	3
2.	25	9.4	494	3
3.	25	11.0	214	0
4.	25-29-25	9.4	935	3
5.	20-29-20	9.4	2243	12
6.	20-29-20	11.0	783	9

<sup>a</sup> Where more than one temperature is given, the values correspond to the embryonic, larval and pupal stages respectively.

<sup>b</sup> Includes only those mutants with a viability of less than 5%, or a developmental delay of more than three days.



TABLE 4

## GENERAL PROPERTIES OF THE NUTRITIONAL MUTANTS

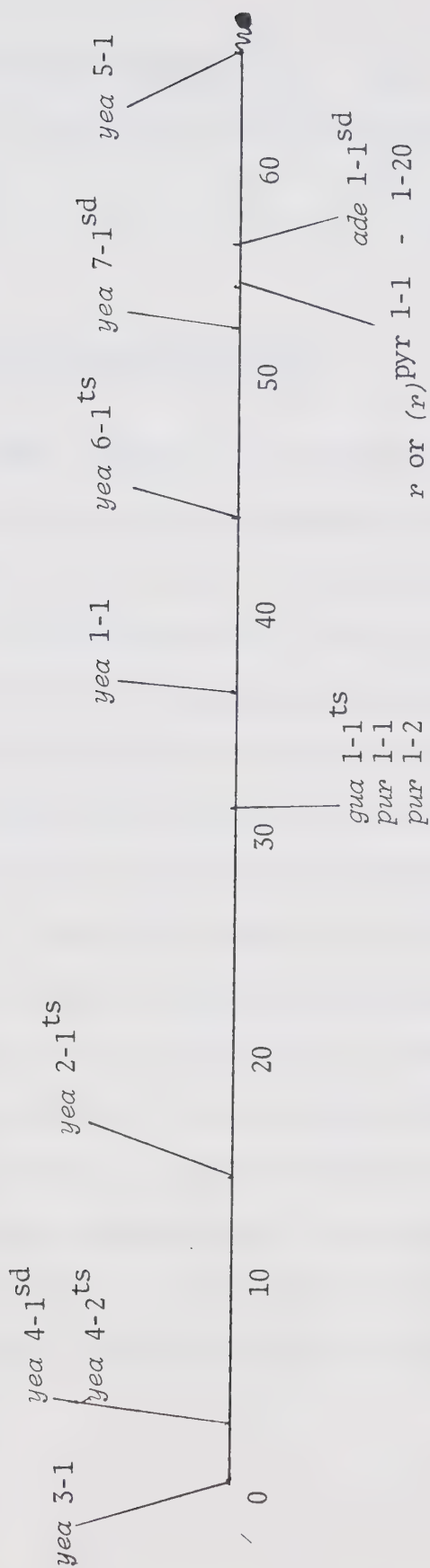
RNA SUPPLEMENTABLE						NON-RNA SUPPLEMENTABLE (Putative Auxotrophs)					
PURINE			PYRIMIDINE								
T.S. <sup>a</sup>	Non T.S. <sup>a</sup>		T.S. <sup>a</sup>		Non T.S. <sup>a</sup>	T.S. <sup>a</sup>		Non T.S. <sup>a</sup>			
<sup>b</sup> Delay	Lethal	<sup>b</sup> Delay	Lethal	<sup>b</sup> Delay	<sup>b</sup> Delay	Lethal	<sup>b</sup> Delay	Lethal	<sup>b</sup> Delay	Lethal	Lethal
0	1	1	2	0	4	0	0	16	2	3	3

<sup>a</sup>T.S. = Temperature Sensitive. For the purposes of this study a t.s. mutation has been defined as one which does not comply with the mutant criteria (5% viability or greater than three day developmental delay) at 25°C, but does at 29°C.

<sup>b</sup>Delay = three or more days.



FIGURE 3 MAP POSITIONS OF X-LINKED AUXOTROPHS<sup>a,b</sup>



a. See Appendix for calculations

b. Superscript Abbreviations: sd - slow developing

ts - temperature sensitive





$29^{\circ}$ , whereas the remainder do not. The latter six will not be discussed in detail here since they are described elsewhere (Falk and Nash, 1972).

## 2. PRELIMINARY CHARACTERIZATION

After isolation and confirmation, the first step in characterization was the RNA supplementability of a mutant effect. Those which did not respond to RNA were not further characterized with respect to supplementation and have been designated "putative auxotrophs". Eight putative auxotrophs were isolated and the auxotrophy of five of these is sensitive to temperature (Table 4).

Those which responded to RNA were further tested with individual purine and pyrimidine nucleosides. These mutants fit into two classes, those which respond to at least one purine nucleoside (purine auxotrophs) and those which respond to both pyrimidine nucleosides (pyrimidine auxotrophs). There are four purine auxotrophs, one of which is temperature sensitive. Of the 20 pyrimidine auxotrophs, four are temperature sensitive (Table 2). Three of the 32 mutants are characterized by a developmental delay of at least three days on the restrictive medium. One is purine nucleoside supplementable, the supplementation of the other two is as yet undefined.



### 3. THE PUTATIVE AUXOTROPHS

The eight putative auxotrophs were mapped (Figure 3, also see Appendix), and those with similar map positions, tested for complementation (Table 5). Seven loci have been identified, all except one of which are represented by one allele (Figure 3). The mutants are of three types: those which are lethal on Sang's at 25° (*yea*); those which are lethal on Sang's at 29° (*yea*<sup>ts</sup>); and those which develop slowly on Sang's (*yea*<sup>sd</sup>).

#### a. The *yea* Mutants

Three mutant strains require yeast for survival at 25°C but show little or no response to RNA (Table 6). These putative auxotrophic mutations map at quite distinct loci, *yea* 3-1, 0.7; *yea* 1-1, 36.6; *yea* 5-1, 66. Their biochemical defect has not been characterized.

All three of these mutant strains are unsupplementable at a temperature extreme. *yea* 1-1 is a cold sensitive lethal, the other two, *yea* 3-1 and *yea* 5-1 are heat sensitive (Table 6). Since the temperature sensitivities map as mutations close to or at the same site as the primary nutritional loci (Table 7), it is assumed, there being no evidence to the contrary, that in each case the temperature sensitivity is a pleiotropic effect of the nutritional mutation.

The *yea* 1-1 X chromosome bears a "genetically rescuable" female sterility mutation. Females homozygous for this X chromosome produce no viable offspring unless fertilized by a non-mutant X-bearing



TABLE 5      COMPLEMENTATION ANALYSIS OF PUTATIVE AUXOTROPHS<sup>a</sup>

	<i>yea</i> 4-2 <sup>ts</sup>	<i>yea</i> 4-1 <sup>sd</sup>	<i>yea</i> 3-1
1. <i>yea</i> 3-1	+ (37-53)	+ (34-44)	- (0-9)
<i>yea</i> 4-1 <sup>sd</sup>	- (0-55)	- (1-40)	
<i>yea</i> 4-2 <sup>ts</sup>	- (0-17)		
	<i>yea</i> 7-1 <sup>sd</sup>		
2. <i>yea</i> 6-1 <sup>ts</sup>	+ (6-8)		
<i>yea</i> 7-1 <sup>sd</sup>	- (2-40)		

a

Only those mutations with similar map positions were tested for complementation.

Female progeny of crosses: *yea* 'x' (or 'y') X *yea* 'y' (or 'x')/FM7b were scored. The numbers in brackets represent the number of *yea* 'x'/'yea' 'y' and the number of *yea* 'x' (or 'y')/FM7b female progeny respectively. Larvae were cultured at 29<sup>o</sup>, embryos and pupae at 25<sup>o</sup>C. Progeny of crosses involving *yea* 4-1<sup>sd</sup> were scored only over the first three days of emergence. All mutations had earlier been confirmed as recessive. The data from the reciprocal crosses are combined.





TABLE 6  
SUPPLEMENTATION OF *yea* MUTANTS

MUTANT	SANG'S S (25°C)		SANG'S S + RNA (25°C)		YEAST (25°C)		YEAST (29°C)		YEAST (18°C)	
	NO. OF ♂♂	NO. OF XXY ♀♀	NO. OF ♂♂	NO. OF XXY ♀♀	NO. OF ♂♂	NO. OF XXY ♀♀	NO. OF ♂♂	NO. OF XXY ♀♀	NO. OF ♂♂	NO. OF XXY ♀♀
<i>yea</i> 1-1	0	287	11	128	128	116	c		3	68
<i>yea</i> 3-1	1	427	0	80	68	67 <sup>b</sup>	2	34	c	
<i>yea</i> 5-1	0	400	0	74 <sup>a</sup>	20	18	0	94	c	
Amherst	324	325	250	234	167	186	135	123	111	146

a

Supplemented with 0.1% of each of the four nucleosides rather than RNA

b

Larvae were cultured at 29°, pupae and embryos at 25°

c

Close to normal survival of mutant males, exact numbers were not recorded



TABLE 7 COMPARATIVE MAPPING OF THE AUXOTROPH AND TEMPERATURE SENSITIVE LETHAL PHENOTYPES OF THE *yea* MUTANTS

PHENOTYPE	AMHERST	<i>yea</i> 1-1		<i>yea</i> 3-1		<i>yea</i> 5-1	
	SANG'S (25°C)	SANG'S (25°C)	YEAST (18°C)	SANG'S (25°C)	YEAST (29°C)	SANG'S (25°C)	YEAST (29°C)
<i>pn v m wy f</i>	32.1	66.8	58.6	58.1	44.8	57.3	51.7
<i>+ + + + +</i>	33.5	0.2	0.9	0	0.4	<u>7.8</u>	<u>10.8</u>
<i>pn + + + +</i>	5.0	0	0	<u>21.2</u>	<u>28.5</u>	2.0	5.0
<i>+ v m wy f</i>	4.6	13.9	19.8	<u>0</u>	<u>0</u>	12.9	9.2
<i>pn v + + +</i>	.9	0	0	1.2	5.2	0	0
<i>+ + m wy f</i>	2.8	1.9	2.7	0	0	1.2	0.8
<i>pn v m + +</i>	1.1	<u>3.5</u>	<u>4.5</u>	4.6	4.1	0	0
<i>+ + + wy f</i>	1.0	<u>0.3</u>	<u>0.9</u>	0	0	5.1	1.6
<i>pn v m wy +</i>	7.4	12.1	12.6	12.4	13.0	<u>0</u>	<u>0</u>
<i>+ + + + f</i>	5.6	0	0	0	0	12.8	18.3
<i>pn + + + f</i>	0.9	0	0	0.8	3.0	0.8	2.4
<i>+ v m wy +</i>	0	1.1	0	0	0	0	0
<i>pn + + wy f</i>	0.1	0	0	1.2	1.1	0.4	0
<i>+ v m + +</i>	0.1	1.3	0	0.4	0	0	0
Total	822	1158	111	241	270	255	120

Values are expressed as percentage of the total. Females heterozygous for the nutritional mutation and the *pn v m wy f* chromosome were crossed to *pn v m wy f* males. The male offspring were classified according to phenotype. Crosses were performed on Sang's medium without RNA and on yeast medium at a temperature previously shown to be lethal for the strain being mapped. The critical data, showing the interval containing the mutation are underlined. Note that both the *ts* and the auxotrophic effects of *yea* 5-1, map to the right of forked.



TABLE 8                      GENETIC RESCUE OF THE FEMALE STERILITY OF *yea 1-1*

CROSS			NO. OF FEMALE PARENTS	NO. OF PROGENY
MALE PARENT	X	FEMALE PARENT		
Am Or <sup>+</sup> <sup>a</sup>	X	Am Or <sup>+</sup> <sup>a</sup>	14	465
<i>yea 1-1</i>	X	<i>yea 1-1</i>	20	0
Am Or <sup>+</sup> <sup>a</sup>	X	<i>yea 1-1</i>	12	137 (All female)

<sup>a</sup> Wild type (Amherst-Oregon, see Materials and Methods)

Two males and two virgin females less than one day old were placed in shell vials. Parents were discarded on the eighth day.



TABLE 9

MAPPING OF THE AUXOTROPH AND FEMALE  
STERILITY PHENOTYPES OF *yea 1-1*

PHENOTYPE	PERCENTAGE EXPECTED <sup>a</sup> TO CARRY THE AUXO- TROPHIC MUTATION	NO. CHROMOSOMES TESTED FOR STERILITY	NO. BEARING ♀ STERILE MUTATION
<i>pn v + + +</i>	100	5	5
<i>pn v m + +</i>	7	7	0

a

See Table 6

Females heterozygous for *yea 1-1* and *pn v m wy f* were crossed to *pn v m wy f* males. *pn v + + +* and *pn v m + +* males were individually crossed to FM7b/*yea 1-1* females. The non-FM7b-bearing females from each culture were checked for fertility by crossing to *yea 1-1* males.





TABLE 10

SUPPLEMENTATION OF *yea<sup>ts</sup>* MUTANTS

MUTANT	SANG'S (20°C)		SANG'S (29°C)		SANG'S + RNA (29°C)		YEAST (29°C)	
	NO. OF ♂	NO. OF XXY ♀♀	NO. OF ♂	NO. OF XXY ♀♀	NO. OF ♂	NO. OF XXY ♀♀	NO. OF ♂	NO. OF XXY ♀♀
<i>yea</i> 2-1 <sup>ts</sup>	88	117	8	377	1	37	107	158
<i>yea</i> 6-1 <sup>ts</sup>	24	125	25	1047 <sup>a</sup>	19	233 <sup>a,b</sup>	65	53 <sup>a</sup>
<i>yea</i> 4-2 <sup>ts</sup>	131	184	5	147 <sup>a</sup>	7	68 <sup>a</sup>	148	205 <sup>a</sup>
Amherst	127	127	412	365	361	40	135	123

<sup>a</sup> Larvae were cultured at 29°C, pupae and embryos at 20°C.<sup>b</sup> Supplemented with 0.1% of each of the four nucleosides rather than RNA.



sperm (Table 8). All resulting offspring are female. This mutation, like the auxotrophic and cold sensitive lethal mutation(s), maps very near *miniature* (Table 9) and thus it seems likely that the female sterility is a third pleiotropic effect of the same mutation.

#### b. The *yea*<sup>ts</sup> Mutants

Three mutants require yeast for survival at 29°C, but not at lower temperatures (Table 10). They are *yea* 4-2<sup>ts</sup>, *yea* 2-1<sup>ts</sup>, and *yea* 6-1<sup>ts</sup> at map positions 5, 16, and 45 respectively (Figure 3 and Appendix). Two of the strains, *yea* 4-2<sup>ts</sup> and *yea* 2-1<sup>ts</sup> are approximately 75% viable on defined medium at 20°C, the other *yea* 6-1<sup>ts</sup> is 20% viable. The viability of the three strains at 29° is not influenced by nucleosides (Table 10).

*yea* 4-2<sup>ts</sup> and *yea* 6-1<sup>ts</sup> were isolated in the temperature shift screens designed for the isolation of larval *ts* auxotrophs. As mentioned in the Materials and Methods, one of the goals of such a screen was the isolation of *ts* auxotrophic mutations in genes unconditionally required during the unsupplementable embryo or pupal stages and thus not normally amenable to auxotrophic analysis. Such mutations would be lethal if the pupal and embryo stages were kept at 29°. Neither of these mutants is *ts* lethal and they do not, therefore, fit into this category.

*yea* 2-1<sup>ts</sup> was isolated in a constant temperature screen (screen 1, Table 3) as a semi-lethal, slow developing mutant at 25°. The temperature sensitive period of this mutant has not been determined.



### c. The *yea*<sup>sd</sup> Mutants

Several mutants have been isolated which show reduced development rate on defined medium, but develop at a normal rate on yeast-sucrose medium. One of these is adenosine supplementable and will be dealt with later. Two others, *yea* 4-1<sup>sd</sup> and *yea* 7-1<sup>sd</sup> are unresponsive to RNA and will be described here. These mutants map at 3 and 53 respectively (Figure 3 and Appendix). At 29°<sub>0</sub>, *yea* 4-1<sup>sd</sup> males do not eclose until five or more days after eclosion of their  $\overline{XX}/Y$  sisters but they are completely viable (Table 11). *yea* 7-1<sup>sd</sup> has even greater delay as well as a yeast supplementable decreased viability (20%) (Table 12). Both mutations are temperature sensitive. The delay and, in the case of *yea* 7-1<sup>sd</sup>, the semi-lethality are less extreme at lower temperatures (Table 11, Table 12).

*yea* 4-1<sup>sd</sup> is an allele of *yea* 4-2<sup>ts</sup>. Although both are temperature sensitive, *yea* 4-2<sup>ts</sup> is distinguishable from its allele by lethality on the defined medium at high temperatures (Table 10).

In summary, the putative auxotrophs represent seven apparently distinct loci. The expression of all mutations is influenced by temperature and one of the mutations is also associated with a genetically rescuable, female sterility effect.



TABLE 11  
SUPPLEMENTATION OF *yea* 4-1<sup>sd</sup>

MEDIUM		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	X X X	DAY 12
SANG'S (Unsupp.)	♂♂	0	0	0	1	11	67	X X X	241
	XXY ♀♀	86	176	206	216	224	226	X X X	228
SANG'S (Unsupp.) (25°C)	♂♂	0	0	9	39	109	181	X X X	230
	XXY ♀♀	42	114	172	214	218	218	X X X	218
SANG'S (+ RNA)	♂♂	0	0	0	3	20	54	X X X	98
	XXY ♀♀	12	34	37	48	50	52	X X X	56
YEAST	♂♂	25	147	265	320	326	326	X X X	326
	XXY ♀♀	106	221	255	271	275	275	X X X	275

Larvae were cultured at 29°, embryos and pupae at 20°, except where otherwise indicated. Day 1 represents the first day of adult emergence in a culture. All values are cumulative totals.





TABLE 12  
SUPPLEMENTATION OF *yea* 7-1<sup>sd</sup>

MEDIUM		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	x x x	DAY 12
SANG'S	♂♂	0	0	0	0	0	3	x x x	31
(Unsupp.)	XXY ♀♀	46	108	132	151	162	170	x x x	172
SANG'S	♂♂	0	2	14	49	77	98	x x x	118
(Unsupp.) (20°C)	XXY ♀♀	20	49	78	91	96	96	x x x	97
SANG'S	♂♂	0	0	0	0	1	4	x x x	20
( + RNA )	XXY ♀♀	41	51	58	61	63	66	x x x	68
YEAST	♂♂	38	82	100	108	119	119	x x x	119
	XXY ♀♀	72	111	123	125	128	128	x x x	128
								x x x	

Larvae were cultured at 29°, embryos and pupae at 20°, except where otherwise indicated. Day 1 represents the first day of adult emergence in a culture. All values are cumulative totals.



#### 4. THE PURINE AUXOTROPHS

At least two purine loci have been identified. One of these is represented by only one allele (*ade 1-1<sup>sd</sup>*) and it is located just proximal to *forked* at about 57 (Figure 3, see also Appendix). The other locus is complex in nature and maps at about 31 (Figure 3, see also Appendix). Three mutations map in the latter region, *gua 1-1<sup>ts</sup>*, *pur 1-1*, and *pur 1-2*. *pur 1-1* and *pur 1-2* do not complement (Table 13a). *gua 1-1<sup>ts</sup>* and *pur 1-1* heterozygotes are delayed in eclosion, although they have high viability, and heterozygotes between *gua 1-1<sup>ts</sup>* and *pur 1-2* show almost complete complementation, even with respect to development time (Table 13b).

Each of the purine auxotrophs has unique characteristics and these will be described separately.

##### a. *ade 1-1<sup>sd</sup>*

*ade 1-1<sup>sd</sup>* males eclose about three days later than their  $\overline{XX}/Y$  sisters on the unsupplemented medium. Pyrimidines have no effect on this delay (Table 14). Guanosine does not affect the development time, but it does lower the viability (Table 14) indicating an enhancement of the mutant effect. In contrast, adenosine suppresses the mutant phenotype. *ade 1-1<sup>sd</sup>* males have normal viability and develop at close to a normal rate in the presence of this purine.

Although only one allele has been isolated at this locus, this is probably not a true indication of its mutation rate. *ade 1-1<sup>sd</sup>* was isolated in the less stringent screen 1 (Table 3). In later



TABLE 13      COMPLEMENTATION ANALYSIS OF *gua* 1 and *pur* 1 AUXOTROPHS

a. On the basis of survival.

	<i>pur</i> 1-2	<i>pur</i> 1-1	<i>gua</i> 1-1 <sup>ts</sup>
<i>gua</i> 1-1 <sup>ts</sup>	+ (19-19)	± (55-74)	- (5-22)
<i>pur</i> 1-1	- (0-28)	- (0-24)	

Female progeny of crosses, strain 'x' (or 'y') males X strain 'y' (or 'x') / FM7b females were scored. The numbers in brackets represent the number of strain 'x' / strain 'y' and the number of strain 'x' (or 'y') / FM7b female progeny, respectively. Larvae were cultured at 29°C. All mutations had earlier been confirmed as recessive. The data from the reciprocal crosses are combined.

continued on next page



TABLE 13 - CONTINUED

COMPLEMENTATION ANALYSIS OF *gua* 1 and *pur* 1 AUXOTROPHS

b. On the basis of growth rate.

'x'	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5	
	Genotype <sup>a</sup>		Genotype <sup>a</sup>		Genotype <sup>a</sup>		Genotype <sup>a</sup>		Genotype <sup>a</sup>	
	A	B	A	B	A	B	A	B	A	B
<i>gua</i> 1-1 <sup>ts</sup>	0	5	0	17	2	20	3	22	5	22
<i>pur</i> 1-1	1	6	2	30	14	41	28	43	31	44
<i>pur</i> 1-2	0	2	5	12	11	17	14	19	19	19
Amherst	18	26	51	60	64	75	73	86	76	89

<sup>a</sup> A = 'x'/*gua* 1-1<sup>ts</sup> B = FM7/*gua* 1-1<sup>ts</sup>

Female progeny of the cross, 'x' males X FM7b/*gua* 1-1<sup>ts</sup> females were scored daily. Parents had been allowed to oviposit for 24-48 hours. Day 1 represents the first day of eclosion for a culture. Larvae were cultured at 29°. All values are cumulative totals.





TABLE 14

SUPPLEMENTATION OF *ade* 1-1

MEDIUM		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8 (Final)
SANG'S	♂♂	0	6	27	106	211	286	352	384
(Unsupp.)	XXY ♀♀	124	242	362	417	421	422	424	424
SANG'S	♂♂	38	133	171	175	175	175	175	175
+ ADENOSINE	XXY ♀♀	53	121	141	146	146	146	146	146
SANG'S	♂♂	0	0	5	11	31	49	52	52
+ GUANOSINE	XXY ♀♀	24	59	85	102	109	116	116	116
SANG'S	♂♂	0	0	13	38	68	82	82	82
+ URIDINE	XXY ♀♀	28	65	89	100	102	103	103	103
SANG'S	♂♂	0	1	7	33	59	72	72	72
+ CYTIDINE	XXY ♀♀	13	55	101	104	104	104	104	104

Cultures were maintained at 25°C. Supplements are added at a concentration of 0.1%. Values are expressed as cumulative totals. No flies emerged after Day 8.



TABLE 15 SUPPLEMENTATION OF *gua* 1 and *pur* 1 MUTANTS

MUTANT		UNSUPP. (20°C)	UNSUPP. (25°C)	UNSUPP. (29°C)	+ ADENOSINE	+ GUANOSINE	+ URIDINE	+ CYTIDINE
<i>gua</i> 1-1 <sup>ts</sup> <sup>a</sup>	♂♂	115	112	6	1	69	0	5
	XXY ♀♀	144	217	152	49	71	34	64
<i>pur</i> 1-1 <sup>b</sup>	♂♂	-	27	-	94	188	43	20
	XXY ♀♀	-	390	-	163	263	279	241
<i>pur</i> 1-2 <sup>b</sup>	♂♂	-	9	-	55	142	1	2
	XXY ♀♀	-	283	-	25	163	181	240
Amherst <sup>b</sup>	♂♂	122	643	412	204	157	149	87
	XXY ♀♀	127	666	365	231	214	133	94

<sup>a</sup> Supplementation tests carried out at 29°C.

<sup>b</sup> Supplementation tests carried out at 25°C.

When added, the concentration of a supplement was 0.1%.



TABLE 16      COMPARATIVE LINKAGE STUDIES ON THE NUTRITIONAL  
AND FEMALE STERILITY MUTATIONS OF THE *gua 1-1<sup>ts</sup>*  
CONTAINING X-CHROMOSOME

PHENOTYPE	PERCENTAGE EXPECTED <sup>a</sup> TO BEAR <i>gua 1-1<sup>ts</sup></i>	NO. CHROMOSOMES TESTED FOR FEMALE STERILITY	NO. BEARING FEMALE STERILE MUTATION
<i>pn + + + +</i>	83	7	7
<i>pn v + + +</i>	0	6	6

<sup>a</sup> Calculated from mapping data shown in Appendix.

Females heterozygous for *gua 1-1<sup>ts</sup>* and *pn v m wy f* were crossed to *pn v m wy f* males. *pn + + + +* and *pn v + + +* males were individually crossed to FM7b/*gua 1-1<sup>ts</sup>* females. The non-FM7b-bearing females from each culture were checked for fertility by crossing to their brothers.



TABLE 17      ECLOSION PATTERN OF *pur* 1-1 and *pur* 1-2 ON ADENOSINE  
OR GUANOSINE SUPPLEMENTED MEDIUM

a. + Adenosine

STRAIN		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
<i>pur</i> 1-1	♂	5	10	16	51	66	90	94
	XXY ♀	75	138	162	162	163	163	163
<i>pur</i> 1-2	♂	22	48	53	55	55	55	55
	XXY ♀	8	16	24	25	25	25	25
Amherst	♂	28	130	186	202	204	204	204
	XXY ♀	87	185	222	229	231	231	231

b. + Guanosine

<i>pur</i> 1-1	♂	21	81	136	175	188	188	188
	XXY ♀	38	136	220	269	283	283	283
<i>pur</i> 1-2	♂	46	99	134	142	142	142	142
	XXY ♀	29	83	147	163	163	163	163
Amherst	♂	18	88	130	149	157	157	157
	XXY ♀	29	108	171	203	214	214	214

Tests were carried out at 25°C on Sang's medium with 0.1% adenosine or guanosine. Day 1 represents the first day of adult emergence. Values are expressed as cumulative totals. Day 7 represents the final total, since it was the last day of emergence.





screens, cultures were not saved for retesting unless the delay was greater than four days, thus a mutant with the properties of *ade 1-1<sup>sd</sup>* would not have been detected.

*ade 1-1<sup>sd</sup>* is not sensitive to temperature. It has been checked on the defined medium to see if the auxotroph phenotype would be more extreme at high temperatures and also on yeast-sucrose at 29° and 20° as a check for *ts* lethality. Both tests were negative.

b. *gua 1-1<sup>ts</sup>*

*gua 1-1<sup>ts</sup>* was originally isolated at 25°C (screen 1, Table 3), at which temperature it has a developmental delay of one day and approximately 50% viability. Subsequent testing showed it to be almost lethal on defined medium at 29°C (Table 15). The mutant is supplementable by guanosine but not by adenosine.

The *gua 1-1<sup>ts</sup>* X chromosome carries a female sterile mutation. Mapping data, however show that this phenotype is the result of a mutation elsewhere on the chromosome (Table 16). In contrast to the nutritional mutation, the female sterility maps proximal to *vermilion*. In addition, the *gua 1-1<sup>ts</sup>* chromosome has a mutation resulting in orange eyes, but this also is distinct from the purine auxotrophy. This mutation maps near the distal tip of the chromosome and is an allele of the *white* locus.

c. *pur 1-1*

Like *gua 1-1<sup>ts</sup>*, the lethal effect of the *pur 1-1* mutation is



guanosine supplementable (Table 15). However, unlike the former mutant, *pur* 1-1 is partially remediable by adenosine. The adenosine-rescued males are delayed by several days (Table 17).

*pur* 1-1 is a relatively "leaky" mutation. Viability at 25°C is about 7%. Flies which are successful in completing development appear to be approximately half the normal body size.

d. *pur* 1-2

*pur* 1-2 is the most extreme of the three mutations in this region. It has about 3% viability at 25°C in the absence of purines (Table 15). Unlike *pur* 1-1, the escapers are not noticeably small in size. Furthermore, the lethality of *pur* 1-2 is fully supplemented by either adenosine or guanosine. No developmental delay is found with adenosine (Table 17).

To summarize the latter three mutations: they are all guanosine remediable; one (*gua* 1-1<sup>ts</sup>) is not affected by adenosine, another is fully supplemented by it (*pur* 1-2), and the third is intermediate in this respect; the two which show some adenosine supplementation do not complement (*pur* 1-1 and *pur* 1-2), but both do complement (at least to some extent) with *gua* 1-1<sup>ts</sup>.



## 5. THE PYRIMIDINE AUXOTROPHS

Twenty pyrimidine dependent strains were isolated. Of these, 16 are auxotrophic at 25°C and their response to nucleoside supplementation is shown in Table 18. The four other mutations do not fit the pre-established criteria for auxotrophy (<5% viability) at 25°C, but do (or, in one case, almost do) at 29°C. These mutations are described in Table 19. All twenty map in the same region (52-56) of the X chromosome (Figure 3, see Appendix for detailed data) suggesting allelism, but complementation testing is difficult since all mutations show some degree of dominance with respect to pyrimidine requirements (Table 23, col 2). Since a number of the mutants have abnormal wings, an effect which is not dominant, complementation analysis has been possible at this level. None of the eleven mutations resulting in mutant wings complements (Table 20) with the "non-complementing" (see below) *rudimentary* allele,  $r^{45}$  (Carlson, 1971). Therefore, these eleven are all part of the *rudimentary* locus ( $r$ ; 1, 54.5), recently established as a site for pyrimidine auxotrophs (Norby, 1970).

Carlson (1971) has shown that there is an excellent correlation between complementation pattern and intra-locus map position of *rudimentary* alleles. A sample of four mutations was taken from the collection he studied:  $r^1$  does not complement with a number of alleles mapping near it at the distal end of the locus;  $r^{11}$  and  $r^{20}$  will not complement with alleles in the central region of the locus; finally,  $r^{29}$  is a representative of a third group and will not complement with most alleles mapping in the proximal part of the locus.



TABLE 18

SUPPLEMENTATION OF PYRIMIDINE AUXOTROPHS

MUTATION	UNSUPP.		+ ADENOSINE <sup>a</sup>		+ GUANOSINE <sup>a</sup>		+ URIDINE		+ CYTIDINE	
	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀
a. Mutants Only Partially Supplemented by .1% Pyrimidine										
r <sub>1</sub> pyr 1-8	0	483					18	100	30	183
r <sub>1</sub> pyr 1-9	0	286					6	33	14	95
r <sub>1</sub> pyr 1-14	0	393					56	74	67	95
r <sub>1</sub> pyr 1-15	0	368					14	51	21	87
r <sub>1</sub> pyr 1-19	0	621	0	27	0	22	26	53	47	123
r <sub>1</sub> pyr 1-20	0	286	0	48	0	270	37	203	28	177
b. Non-Leaky Mutations Which are Fully Supplemented by Pyrimidines (.1%)										
r <sub>1</sub> pyr 1-16	0	312			0	51	118	86	34	49
r <sub>1</sub> pyr 1-11	0	310	0	29	0	28	53	32	61	50
r <sub>1</sub> pyr 1-12	0	354	0	24	0	111	61	38	69	50
r <sub>1</sub> pyr 1-13	0	477	0	36	0	26	22	29	54	51
(r <sub>1</sub> )pyr 1-16	0	231					62	58	27	39
r <sub>1</sub> pyr 1-17	0	332	0	49	0	118	72	30	104	82

continued on next page





TABLE 18 - CONTINUED

## SUPPLEMENTATION OF PYRIMIDINE AUXOTROPHS

MUTATION	UNSUPP.		+ ADENOSINE <sup>a</sup>		+ GUANOSINE <sup>a</sup>		+ URIDINE		+ CYTIDINE	
	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀

c. Leaky Mutations Which are Fully Supplemented by Pyrimidines (.1%)

( <i>r</i> )pyr 1-1	6	240	17	212	21	270	305	278	154	155
( <i>r</i> )pyr 1-2	34	851	17	120	10	173	190	187	260	222
( <i>r</i> )pyr 1-4	1	601	0	38	0	69	65	55	147	123
( <i>r</i> )pyr 1-20	25	492	4	28	1	39	139	91	277	205

<sup>a</sup> Only a random sample of strains which had, by complementation, been shown to be part of the *rudimentary* locus were checked for purine supplementation. All tests were carried out at 25°C.



TABLE 19

## SUPPLEMENTATION OF TEMPERATURE SENSITIVE PYRIMIDINE AUXOTROPHS

MUTATION	UNSUPP. 20°C		UNSUPP. 25°C		UNSUPP. 20-29-20°C		+ ADENOSINE 20-29-20°C		+ GUANOSINE 20-29-20°C		+ URIDINE 20-29-20°C		+ CYTIDINE 20-29-20°C	
	♂♂	XXY♀	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀	♂♂	XXY♀♀
( <sup>r</sup> )pyr 1-3 <sup>ts</sup>	c	c	26	319	0	66 <sup>a</sup>	5	16 <sup>b</sup>	10	55 <sup>b</sup>	111	101 <sup>b</sup>	110	76 <sup>b</sup>
( <sup>r</sup> )pyr 1-5 <sup>ts</sup>	123	350	147	842	30	505	0	24	0	59	22	70	114	141
( <sup>r</sup> )pyr 1-6 <sup>ts</sup>	43	295	56	711	2	414	0	79	0	74	120	95	55	50
( <sup>r</sup> )pyr 1-7 <sup>ts</sup>	22	137	151	651	1	141	0	57	1	201	121	102	87	90

a 25-29-25°C; b 25°C; c not determined

When added, the concentration of a supplement was 0.1%. The temperatures for individual tests were as indicated. Where more than one temperature is given the three values refer to the embryonic, larval and pupal stages respectively.



TABLE 20  
COMPLEMENTATION ANALYSIS OF PYRIMIDINE  
AUXOTROPH/ $r^{45}$  HETEROZYGOTES

MUTATION	HEMIZYGOTE VIABILITY <sup>a</sup> (%)	WING SCORE $r^{pyr^+x}/r^{pyr^+x}$	WING SCORE $r^{pyr^+x}/r^{45}$
$r^{pyr}$ 1-19	0	1.0	not checked
$r^{pyr}$ 1-8	0	1.1	1.3
$r^{pyr}$ 1-15	0	1.1	1.8
$r^{pyr}$ 1-14	0	1.3	1.6
$r^{pyr}$ 1-9	0	1.3	2.1
$r^{pyr}$ 1-18	0	1.3	2.2
$r^{pyr}$ 1-12	0	2.2	2.0
$r^{pyr}$ 1-13	0	3.0	2.7
$r^{pyr}$ 1-17	0	3.0	3.0
$r^{pyr}$ 1-10	0	3.1	2.1
$r^{pyr}$ 1-11	0	3.4	3.0
( $r$ ) $^{pyr}$ 1-16	0	4.0	4.0
( $r$ ) $^{pyr}$ 1-4	<1	4.0	3.2
( $r$ ) $^{pyr}$ 1-1	3	4.0	4.0
( $r$ ) $^{pyr}$ 1-2	4	4.0	4.0
( $r$ ) $^{pyr}$ 1-20	5	4.0	4.0
( $r$ ) $^{pyr}$ 1-3 <sup>ts</sup>	8	4.0	4.0
( $r$ ) $^{pyr}$ 1-6 <sup>ts</sup>	8	4.0	4.0
( $r$ ) $^{pyr}$ 1-5 <sup>ts</sup>	18	4.0	4.0
( $r$ ) $^{pyr}$ 1-7 <sup>ts</sup>	23	4.0	4.0
Amherst	100	4.0	4.0
$r^{45}$			2.4

<sup>a</sup> Data taken from Table 18 or 19 (unsupplemented medium).

All data are from experiments carried out at 25°C.

10 ( $r$ ) $^{pyr}$   $x$  /  $r^{45}$  and 10 ( $r$ ) $^{pyr^+x}$  / ( $r$ ) $^{pyr}$   $x$  females were scored for the degree of *rudimentary* wing effect as described in Materials and Methods. A score of 1 represents extreme *rudimentary* wings, a score of 4 represents wild type wings.



TABLE 21 COMPLEMENTATION OF *RUDIMENTARY*-WING PYRIMIDINE AUXOTROPHS WITH FOUR PREVIOUSLY LOCALIZED *RUDIMENTARY* MUTATIONS

MUTATION	WING SCORE <sup>a</sup> OF HOMOZYGOUS FEMALE	COMPLEMENTATION WITH:			
		$r^1$	$r^{11}$	$r^{20}$	$r^{29}$
$r^{pyr} 1-19$	1.0	-	-	-	-
$r^{pyr} 1-8$	1.1	-	-	-	-
$r^{pyr} 1-15$	1.1	+	-	-	+
$r^{pyr} 1-14$	1.3	+	-	-	+
$r^{pyr} 1-9$	1.3	-	-	-	-
$r^{pyr} 1-18$	1.3	+	-	-	+
$r^{pyr} 1-12$	2.2	+	-	-	+
$r^{pyr} 1-13$	3.0	+	-	-	+
$r^{pyr} 1-17$	3.0	+	-	-	+
$r^{pyr} 1-10$	3.1	+	-	-	+
$r^{pyr} 1-11$	3.4	+	-	-	+

<sup>a</sup> Data taken from Table 20.

$r^y/r^{pyr}x'$  female progeny of the cross  $r^{pyr}x' \times ClB/r^y$  were scored for the presence or absence of *rudimentary* wings. All tests were carried out at 25°C.





TABLE 22      EFFECT OF PYRIMIDINE NUCLEOSIDES AND THEIR PRECURSORS  
ON THE SURVIVAL OF (*r*)pyr 1-1

SUPPLEMENT	♂♂	XXY ♀♀
None	6	240
+ Carbamyl <sup>a</sup> Phosphate	6	116
+ Carbamyl Aspartate	88	191
+ Dihydro-orotate	111	227
+ Orotate	115	187
+ Uridine	305	278
+ Cytidine	154	155

All supplements were added at a concentration of 4.0 mM to Sang's medium without RNA. Cultures were maintained at 25°C.

- <sup>a</sup> It is unlikely that carbamyl phosphate would enter cells without modification, due to the phosphate part of the molecule.



TABLE 23

## THE PATTERN OF MUTANT PHENOTYPES OF PYRIMIDINE AUXOTROPHS

MUTANT	HETEROZYGOTE VIABILITY (%)	HEMIZYGOTE VIABILITY (%)	WING SCORE	SUPPLEMENTATION (0.1% pyr nucleoside)	FERTILITY
<i>r</i> <sup>+</sup> pyr 1-14	5 (4.7,5.7)	0	1.3	Partial	0
<i>r</i> <sup>+</sup> pyr 1-19	6 (5.4,5.8)	0	1.0	Partial	0
<i>r</i> <sup>+</sup> pyr 1-8	6 (1.8,7.8)	0	1.5	Partial	0
<i>r</i> <sup>+</sup> pyr 1-15	6 (3.2,9.4)	0	1.5	Partial	0
<i>r</i> <sup>+</sup> pyr 1-9	11 (9.7,14.0)	0	1.5	Partial	0
<i>r</i> <sup>+</sup> pyr 1-18	12 (5.6,20.5)	0	1.7	Partial	0
<i>r</i> <sup>+</sup> pyr 1-12	13 (10.7,14.8)	0	2.5	Complete	0
<i>r</i> <sup>+</sup> pyr 1-17	18 (14.0,28.0)	0	3.1	Complete	0
<i>r</i> <sup>+</sup> pyr 1-10	24 (19.4,33.2)	0	3.0	Complete	0
<i>r</i> <sup>+</sup> pyr 1-13	24 (22.5,25.9)	0	3.2	Complete	<1%
( <i>r</i> )pyr 1-16	30 (35.0,20.3)	0	4.0	Complete	Fertile
<i>r</i> <sup>+</sup> pyr 1-11	41 (42.6,39.3)	0	3.7	Complete	<1%
( <i>r</i> )pyr 1-20	41 (53.4,34.4)	5	4.0	Complete	Fertile
( <i>r</i> )pyr 1-2	47 (47.9,45.8)	4	4.0	Complete	Fertile
( <i>r</i> )pyr 1-3 <sup>ts</sup>	47 (70.0,28.1)	8	4.0	Complete	Fertile
( <i>r</i> )pyr 1-6 <sup>ts</sup>	48 (49.8,51.6)	8	4.0	Complete	Fertile
( <i>r</i> )pyr 1-1	53 (60.8,43.3)	3	4.0	Complete	Fertile
( <i>r</i> )pyr 1-4	59 (55.7,64.2)	<1	4.0	Complete	Fertile
( <i>r</i> )pyr 1-5 <sup>ts</sup>	62 (64.9,60.1)	18	4.0	Partial	Fertile
( <i>r</i> )pyr 1-7 <sup>ts</sup>	68 (85.6,57.6)	23	4.0	Complete	Fertile

Incubation temperature was 25°C for all experiments except the supplementation testing of (*r*)pyr 1-7<sup>ts</sup>, (*r*)pyr 1-5<sup>ts</sup>, (*r*)pyr 1-6<sup>ts</sup>. In the latter case the larvae were cultured at 29°C, pupae and embryos at 20°C. Heterozygote viability was determined as described in Materials and Methods. The value reported is the average from two experiments and the values of each of these two experiments are shown in parentheses. Hemizygote viability data is taken from Tables 18 and 19. Wing score was determined by the method described in the Materials and Methods. It is based on the analysis of ten males and ten females. Supplementation data is taken from Tables 18 and 19. Fertility was determined by the method described in the Materials and Methods.



Some alleles will not complement with any *rudimentary* mutations (Fahmy and Fahmy, 1959; Green, 1963). Complementation analysis of these gives no localization information. Three of the present eleven alleles fit into this last category. The other eight all complement with the mutations at the two ends of the locus but not with  $r^{11}$  and  $r^{20}$  (Table 21). This suggests that these eight mutations are all localized around  $r^{11}$  and  $r^{20}$ .

Nine of the pyrimidine auxotrophs have normal wings. This might be indicative of such mutations not being a part of the *rudimentary* locus, but several lines of evidence suggest that this is not the case. Two of the normal wing auxotrophs have essentially zero viability in the absence of pyrimidines<sup>2</sup>,  $(r)^{\text{pyr 1-4}}$  and  $(r)^{\text{pyr 1-16}}$ . When one of these,  $(r)^{\text{pyr 1-4}}$  is heterozygous with  $r^{45}$ , most of the resulting females have *rudimentary* wings (Table 20). In the case of  $(r)^{\text{pyr 1-16}}$  most heterozygotes with  $r^{45}$  have wild type wings, but a few (less than one in ten) have a slight *rudimentary* effect. The remaining seven, all of which are "leaky" auxotrophs have normal wings as heterozygotes with  $r^{45}$ .

One of the normal wing mutants  $(r)^{\text{pyr 1-1}}$  was tested for supplementation with pyrimidine precursors. The results were the same as for previously published studies with *rudimentary* mutations resulting in abnormal wings (Norby, 1970). The second precursor,

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<sup>2</sup> Those mutations which are probably *rudimentary* alleles even though they have normal wings (see text for evidence), are given the designation  $(r)$ , followed by their auxotroph designation as a superscript.



carbamyl aspartate and precursors following it in the pathway are capable of supplementing the mutant effect (Table 22).

Like the abnormal-wing mutants, the mutants with normal wings are partially dominant with respect to auxotrophy. The degree of dominance is not the same for all mutations. Table 23 lists the mutants in order of dominance. It varies, from a heterozygote viability of 5% in the absence of a pyrimidine source to almost 70% (Table 23). The order must not be taken as exact since there is fluctuation between the two experiments. However, it is apparent that the degree of expression of the various mutant phenes of *rudimentary* correlates very closely. The mutations which result in very low heterozygote viability also have the most extreme *rudimentary* wing effect. Such mutations are only partially supplementable with 0.1% pyrimidine nucleosides and are sterile as homozygous females. Those mutants with an intermediate degree of dominance (10 - 40%) also have an intermediate wing effect; they are completely supplemented by 0.1% pyrimidine nucleosides and some are at least partially fertile as females. Lastly, those mutations which are the least dominant are often leaky auxotrophs, have normal wings and are fertile as females.





## DISCUSSION

### 1. EMS AS A MUTAGEN

Chemically, ethyl methane sulfonate (EMS) alkylates guanine to 7-ethylguanine and, with lesser efficiency, adenine to 3-ethyladenine or 1-ethyladenine (Brooks and Lawley, 1961; Lawley and Brooks, 1963). Genetically, EMS induces many GC to AT transitions, as well as lesser numbers of AT to GC transitions, base insertions and deletions, and non-revertible mutations in *Neurospora crassa* (Malling and de Serres, 1968). Similar results with bacteriophage, T4 led to the suggestion that EMS induces pairing errors, 7-ethylguanine with thymine and 3-ethyladenine with cytosine, as well as replication errors at the site of gaps produced by the hydrolysis of 7-ethylguanine (Krieg, 1963).

The greater complexity of higher eukaryote chromosomes as compared to those of lower organisms might result in some basic differences in the chemical nature of EMS-induced mutations. However, it is clear that the majority of EMS induced mutations in *Drosophila* are not associated with chromosome aberrations (Lim and Synder, 1968) and most appear to be single gene mutations (Lifschytz and Falk, 1969; Judd, Shen, and Kaufman, 1972). Exceptions do exist and small deletions, larger deficiencies (Lifschytz and Falk, 1969), an inversion (Vyse, 1969) and a translocation (Grigliatti et al, 1973) have been isolated in EMS-mutation studies.



In the present experiments, the mapping data (Appendix) demonstrate that none of the nutritional mutants are associated with large chromosomal aberrations and is consistent with their having arisen as single point mutational events.

## 2. THE PUTATIVE AUXOTROPHS

Eight mutants with low viability or slow development rate on Sang's medium are supplemented by yeast, but not RNA. Additives other than RNA have not been checked for supplementation, and expression of the mutant phenotypes is, therefore, conditional upon nutritional factors which are as yet undefined.

In addition, mutant expression is conditional upon environmental temperature. Three of the putative auxotrophs are lethal at a temperature extreme, whereas the auxotrophy of the other five is temperature sensitive. In bacteria, mutations which are temperature sensitive can result in the production of a thermolabile protein which becomes non-functional at higher temperatures (Jockusch, 1966). Some evidence exists suggesting that this may also be the case in *Drosophila* (Suzuki, 1970; Camfield and Suzuki, 1972). A basic prediction of this model is that some non-temperature sensitive alleles will exist which will inactivate the protein regardless of temperature. Information on the frequency at which such alleles exist relative to their temperature sensitive counterparts, is not available in the literature. But in *Drosophila* temperature sensitive lethals



over the whole X chromosome occur 6.3% as frequently in response to EMS, as lethals which are not sensitive to temperature (Suzuki et al, 1967). On the basis of these results and the prediction of the current interpretation of temperature sensitivity, the expectation was that non-temperature sensitive *yea* mutations would exist and probably be in the majority. Instead, none were found.

Although it is quite likely that further screening would result in the detection of such mutations, at this point it seems that *yea* mutations which are not sensitive to temperature are relatively rare. Since this finding may contradict a rather basic prediction of the thermolabile protein hypothesis, the possibility that the temperature sensitivity of these mutants has a different basis warrants consideration. It seems possible for example that an organism with heritable metabolic inefficiencies may be inviable at temperatures which approach the limit for viability of wild type flies. This need not necessarily be the result of a decrease in the activity of an enzyme at high temperatures. Rather, it may be the result of an increased demand for a particular metabolite at high temperatures. If such a metabolite is present in short supply even at lower temperatures due to a mutation, the increased demand at higher temperatures could result in lethality. Whether or not this explanation is applicable to the putative auxotrophs will become more clear after the biochemical basis of these mutations is known.

Biochemical information on at least some of these strains will be likely to come through an analysis of the effect of dietary





manipulations on viability. Some of the strains for example, may be blocked in the synthesis of certain vitamins. Examples include cyancobalamine, inositol, or para-aminobenzoic acid and such strains might respond to their addition. The low viability of other strains on defined medium, may be due to defects in aspects of metabolism such as the processing of carbohydrates, or the production of certain steroids or fatty acids. The higher viability of strains such as these on yeast may be a response to a higher concentration of a particular component (for example, sucrose in the case of carbohydrate mutations). Alternatively it might result from the greater variety of components present in yeast, either through the provision of a required end product, or the provision of additional substrates allowing for more leeway in the use of alternative pathways. Attempts to mimic the yeast supplementation of these mutations with specific dietary alterations should provide insight into the nature of the metabolic deficiency of a strain, but there is a second approach which may prove just as useful. This approach is the analysis of effects which *enhance* the expression of a mutation. For example, a mutant in carbohydrate metabolism may become lethal on yeast in the presence of a carbohydrate-specific metabolic inhibitor at a dose which normally does not affect viability. Or, a mutant might become lethal on Sang's at a normally permissive temperature, either in response to an inhibitor or in response to a low concentration of a normal constituent (e.g. cholesterol in the case of steroid mutants).





Some of the *yea* mutants will probably not yield to biochemical analysis. Mutations which affect feeding behaviour or digestion, for example, may prove particularly difficult to analyze. However, problems such as these are associated with almost any collection of mutants and the significant point is that the metabolic deficiency of at least some of these can probably be determined through nutritional alteration experiments as described above.

One of the putative auxotrophs *yea* 1-1 is a genetically rescuable female sterile mutation. Many alleles of the pyrimidine auxotroph, *rudimentary* have the same characteristic, and Norby (1970) has interpreted this result as being indicative of a lethal RNA deficiency in the eggs of females homozygous for this mutation. He suggests that if such eggs are fertilized by an X chromosome bearing a wild type allele, the resulting pyrimidine synthesis occurs in time to replenish the pyrimidine pool before embryonic lethality occurs. It seems likely that an analogous explanation is the most likely interpretation of the *yea* 1-1 mutant as well. Probably, the same mutation which causes the nutritional defect in larvae results in a deficiency in the eggs of homozygous mutant females which is not nutritionally supplementable, but can be replenished through fertilization by a sperm bearing a wild type allele.

Some other mutants are fertile as homozygous females, but generally at a much lower level than wild type. The level of fertility of these mutants is extremely variable and preliminary results suggest that this is due to variation in the availability of nutrients. Such



strains might prove consistently sterile if placed on the adult defined medium of Sang and King (1962). Recent results show that the female sterility of *rudimentary* is compensated by feeding high concentrations of pyrimidines (Bahn, 1970). Future experiments may demonstrate that these mutations, like *rudimentary*, belong to a class of mutants which are conditionally female sterile.

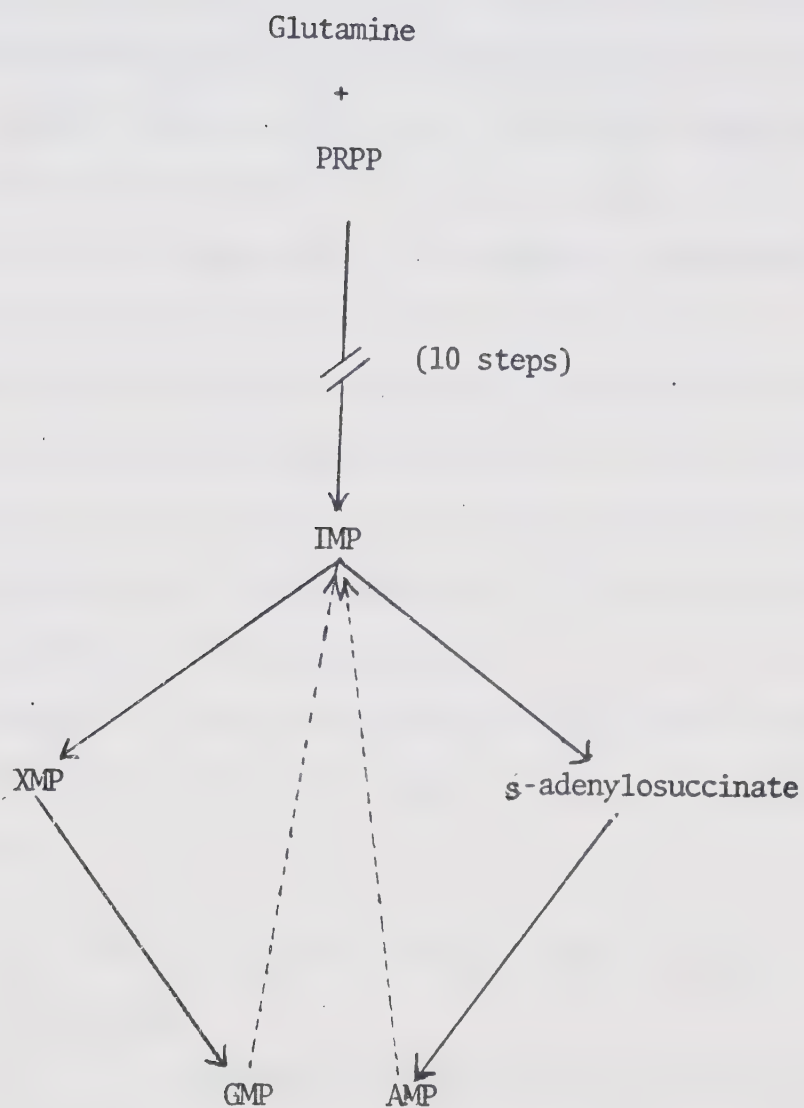
### 3. THE PURINE AUXOTROPHS

Purine biosynthetic mutations in bacteria are generally of three types with respect to adenine and guanine supplementation. Some respond to either purine. These are usually blocked at a step prior to the production of inosine monophosphate (IMP) (Figure 4). Others respond to adenine but not guanine. They are blocked in one of the two steps between IMP and adenine monophosphate (AMP). Finally, there is a group which is guanine supplemented and these are blocked in one of the two steps in the conversion of IMP to GMP (Magasanik, 1957).

In this light, it is interesting that the four purine mutations isolated in these experiments fit into three analogous categories with respect to nucleoside supplementation. Two, *pur* 1-1 and *pur* 1-2 are remediable by adenosine or guanosine. The other two, *ade* 1-1<sup>sd</sup> and *gua* 1-1<sup>ts</sup> are supplemented by only one of these nucleosides. Since the purine biosynthetic pathway in animals is very similar to that of bacteria (Moat and Friedman, 1960), it is possible that these mutations have the same basis as their bacterial



FIGURE 4      PURINE MONONUCLEOTIDE BIOSYNTHESIS



Abbreviations used:

- GMP - Guanosine monophosphate
- IMP - Inosine monophosphate
- PRPP - 5-Phosphoribosyl pyrophosphate
- XMP - Xanthosine monophosphate
- AMP - Adenosine monophosphate



counterparts. Conclusive evidence on this point, is clearly dependent upon a biochemical analysis of these strains.

The proximity of the *gua 1* site to the *pur 1* site is a striking result, particularly in light of the complementation data. On a purine-free medium *gua 1-1<sup>ts</sup>/pur1-1* or 2 heterozygotes survive at close to a normal frequency (Table 13a). If however, development time of such females is examined, it becomes evident that *gua 1-1<sup>ts</sup>/pur 1-1* flies are slow (Table 13b). This implies a functional relationship between these two mutants and evidence for this is strengthened by re-examination of the supplementation data (Table 17). With respect to adenosine supplementation *pur 1-1* differs from its allele, *pur 1-2*. *pur 1-1* mutants are not able to develop at the normal rate on adenosine. Thus, like *gua 1-1<sup>ts</sup>*, *pur 1-1* is inefficient at utilizing adenosine as a supplement.

I believe it would be worthwhile at this point to propose a working hypothesis based on this data. There are three elements to this hypothesis:

- a. *pur 1-1* and *pur 1-2* are mutant at a step prior to IMP synthesis.
- b. *gua 1-1<sup>ts</sup>* is mutant for one of the enzymes converting IMP to GMP.
- c. *pur 1-1* has a slight polar effect, such that it also affects the *gua 1* locus.

The complementation and supplementation data are consistent with all





three elements of this hypothesis. Unfortunately, most of the analysis of these mutants has been carried out very late in the present study. Some of the tests involving supplementation testing with inosine and xanthosine are now in progress, as is a more thorough analysis of complementation. However, even the results of these tests will only become conclusive after an analysis of these strains at the biochemical level.

The close linkage of *gua* 1-1<sup>ts</sup> to the *pur* 1 locus is of particular interest from the standpoint of genetic regulation. In bacteria, several of the purine biosynthetic enzymes are closely linked. For example, the two genes *gua* A and *gua* B, which code for the enzymes catalyzing the two steps in the synthesis of GMP from IMP are linked and co-ordinately controlled in *Escherichia coli* (Nijkamp and DeHaan, 1967). Close linkage and co-ordinate regulation of genes controlling the synthesis of metabolically related enzymes is common in bacteria. In higher organisms however, the situation is not clear due to the small number of loci that have been identified at the enzyme level. In *Drosophila*, the loci of about 20 enzymes have been identified and there is no close linkage between the metabolically related genes (see O'Brien and MacIntyre, 1972). However, recently Norby (1973) has presented preliminary evidence which suggests that the first two enzymes of pyrimidine biosynthesis may be coded for by the *rudimentary* locus (see Introduction). On the basis of the data reported above, it is conceivable that several of the purine biosynthetic enzymes may be closely linked as well.



#### 4. THE PYRIMIDINE AUXOTROPHS

Although the twenty pyrimidine auxotrophs all map in the same region, they have several distinguishable phenotypic differences. Some have wings which are *rudimentary*, some are sterile as homozygous females, and others are phenotypically normal in both of these respects. There are several possible explanations of this result:

- a. the phenotypic differences may reflect mutations at two or more distinct loci.
- b. the normal wings and female fertility of some mutations may be a result of some tissue specific mutations at the *rudimentary* locus.
- c. the phenotypic differences may result from mutations which affect the same locus in basically the same manner. The differences may be a manifestation of the degree to which pyrimidine biosynthesis is blocked.

The data is most consistent with the third suggestion. Table 23 shows that the mutations form a phenotypic gradient. The severity of the pyrimidine auxotrophy appears closely correlated with the severity of the wing defect and the female sterility. Furthermore, the data imply the existence of a series of phenotypic thresholds. For example,  $r^{\text{pyr } 1-12}$  probably provides a level of pyrimidine synthesis slightly above that necessary for complete supplementation with 0.1% pyrimidine nucleosides.  $r^{\text{pyr } 1-13}$  seems to have a level of synthesis which corresponds closely to that necessary for the



production of some viable eggs and  $(r)^{\text{pyr } 1-16}$  is close to the level necessary for the production of normal wings.

Further evidence for these mutations all being part of the *rudimentary* locus, comes from the finding that several of the more extreme (on the basis of hemizygote viability in the absence of pyrimidines) normal-wing strains (  $(r)^{\text{pyr } 1-16}$ ,  $(r)^{\text{pyr } 1-4}$  ) have a slight *rudimentary* wing effect when heterozygous with  $r^{45}$ .

Unfortunately, even though the data strongly suggest that the other normal wing mutations are part of the *rudimentary* locus as well, this has not yet been proven (by complementation analysis), because of the partial dominance of the auxotrophic phenotype.

The partial dominance of auxotrophy at the *rudimentary* locus contradicts the results of an earlier study (Bahn, Norby and Sick, 1971). Some pyrimidine contaminants are known to be present in the Eledon<sup>R</sup> medium which was used in the earlier experiments (Norby, personal communication), and this is the likely reason for this discrepancy.

This partial dominance has at least three possible explanations. The size of the pyrimidine pool of larvae cultured on a pyrimidineless medium may be very near to a threshold such that even a minor decrease in endogenous synthesis affects viability. Alternatively, it is possible that dominance may result from a mutation in a multimeric enzyme (Birnstien and Fisher, 1968). If the sub-units of such an enzyme associate at random, and the presence of only one mutant sub-unit is sufficient to drastically affect enzyme activity, dominance could result. In bacteria





dominance is commonly associated with mutations in a regulatory gene, and it is possible that *rudimentary* may represent a locus of this nature.

There is no data at present to distinguish between these possibilities. The first hypothesis predicts that high levels of enzyme activity will be associated with heterozygotes even though they have a viability as low as 5 to 10% in pyrimidineless medium. The second hypothesis predicts that the level of cross-reacting material (CRM) in the heterozygotes will be high, whereas enzyme activity will be low. Finally, the third hypothesis predicts a low concentration of CRM as well as low enzyme activity in heterozygotes.

From the point of view of mutation rate, the most striking feature of the results is the high recovery of pyrimidine auxotrophs. Using a variety of mutagens, Fahmy and Fahmy (1959a) demonstrated that *rudimentary* mutations are more than twice as frequent as mutations at any other sex-linked morphological locus. Probably only six or seven of the 20 pyrimidine auxotrophs have wing defects severe enough to have been isolated in a morphological screen of the nature of the Fahmys'. Therefore, in view of the extra 13 or 14 mutations isolated at this locus, the mutation rate is remarkably high. The rate of production of mutations at this locus is about five to six times that of morphological mutations at any other locus on the X chromosome.





A high mutation rate of a particular gene may have a variety of causes:

- a. Size, chemical composition, or accessibility to mutagen of the gene.
- b. Peculiar sensitivity of a protein to structural alteration.
- c. Peculiar sensitivity of the organism to a slight change in protein function.

The cause of the high rate of recovery of pyrimidine auxotrophs is not at all clear. The fact that a screen for auxotrophy detected mutations which by previous morphological criteria, would have gone undetected, indicates the auxotrophic phenotype is more sensitive to loss of protein function than normal morphological criteria (possibility c.). This however, is probably not the only cause of the high mutation rate. The data in Table 21 indicate that all eight of the mutations, susceptible to the type of complementation analysis described in the Results section, are in the same region of the locus, and this region only represents about half of the *rudimentary* locus (Carlson, 1971). The finding of many mutations in this region is consistent with the results of the Fahmys (1959b). Since the *rudimentary* locus may code for more than one enzyme (Norby, 1973), this region may correspond to an enzyme particularly susceptible to structural alteration (possibility b.). Furthermore, these results could also be consistent with several of the aspects of possibility a. In light of these results, it seems likely that the high mutation rate



of this locus may not have any single basis, but is probably the result of a number of factors.

## 5. CONCLUSIONS

It would be useful if an analysis of the mutation rate could provide an estimate of the number of potential auxotrophic loci. One approach to this problem has been to analyze the distribution of repeat mutations by means of a Poisson distribution (e.g. Hochman, 1971). This method is not reliable for this data for several reasons. First, about three quarters of the loci are represented by only one allele, and this indicates that we have not yet come close enough to saturating the X chromosome to perform this type of analysis. Secondly, there appears to be a diversity in mutation rate for the auxotrophic loci identified. Just as one locus has been identified which has a very high mutation rate, it is possible that a number of loci will have a very low mutation rate.

Fahmy and Fahmy (1959a) demonstrate that many morphological loci have a mutation rate of less than 1/10 that of *rudimentary* morphological mutations. Since in the present study, six or seven *rudimentary* mutations would have been detected by their criteria, this further suggests that loci are yet to be detected on the X chromosome.

The X chromosome represents about one fifth of the genome of *D. melanogaster*. In the present study, eleven auxotrophic or putative auxotrophic loci were detected. If eleven is taken as being



the minimal number of auxotrophic loci on the X chromosome, and if the assumption is made that such loci are evenly distributed throughout the genome a minimal estimate of the number of such loci in the genome is 55.

Even if the estimate of the number of loci potentially detectable by this technique were reasonable, this still represents only a fraction of the total number of possible nutritionally sensitive loci. The reason for this is that the estimate clearly depends upon the screen used: for example, use of high temperatures during larval development increased the number of mutants recovered by about 50%. Manipulation of the medium by reducing the concentration of particular constituents to sub-optimal levels might likewise increase the recovery of mutants. Indeed, it is possible that some of the conditionally semi-lethal mutants discussed by Falk and Nash (1972) might be lethal if a particular nutrient were in short supply. Furthermore, since Sang's medium contains amino acids not obligately required by *Drosophila*, additional loci might also be identified using Geer's (1965) minimal amino acid medium. Finally, additional nutritionally responsive loci may be identifiable in strains already carrying one mutation. For example, the use of a strain blocked in glucose-6-phosphate dehydrogenase (*zw*; 1,63) which is not lethal on Sang's medium (Falk, unpublished), might reveal mutants in other steps of carbohydrate metabolism, which would not otherwise be detected as nutritional mutants (J. Sparrow, personal communication).



With regard to the characteristics of the auxotrophs which have been isolated, the results indicate that as with bacteria, the analysis of nutritional mutations in *Drosophila* can answer some basic genetical questions. There is preliminary evidence that both a purine and a pyrimidine (Norby, 1973) locus code for more than one enzyme in their respective pathways. If biochemical analysis confirms this, nutritional loci will already have answered a rather basic question with regard to the organization of genes in higher organisms. However, the generality of such a finding will likely only become apparent by the identification and analysis of a large number of biochemically-definable mutations and the nutritional approach may be the best way to get such mutations.





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## A P P E N D I X



TABLE A-1

## RECOMBINATION DATA OF THE MUTANTS

	Amherst	Amherst <sup>a</sup>	Amherst <sup>b</sup>	Amherst <sup>c</sup>	Amherst <sup>d</sup>	yea 3-1	a <sub>yea 4-1<sup>sd</sup></sub>	b <sub>yea 4-1<sup>ts</sup></sub>	c <sub>yea 2-1<sup>ts</sup></sub>
<i>pn v m wy f</i>	37.1	41.9	25.2	34.6	54.5	58.3	66.0	56.9	62.9
<i>+ + + + +</i>	33.3	30.3	34.8	33.8	24.5	0	0	0.6	6.0
<i>pn + + + +</i>	5.0	4.2	12.0	6.6	2.1	21.3	13.7	17.6	7.7
<i>+ v m wy f</i>	4.6	5.2	65.	6.2	3.5	0	1.0	1.2	5.0
<i>pn v + + +</i>	0.9	0.8	1.5	0.7	0.7	1.3	2.9	2.3	1.1
<i>+ + m wy f</i>	2.8	2.7	0.9	1.4	1.4	0	0	0	0.3
<i>pn v m + +</i>	1.1	1.1	2.6	2.0	0.7	4.6	3.9	6.8	3.4
<i>+ + + wy f</i>	1.0	1.0	2.0	1.4	0.7	0	0	0	0
<i>pn v m wy +</i>	7.4	6.6	5.2	8.6	6.9	12.5	12.7	12.3	11.9
<i>+ + + + f</i>	5.6	5.0	6.7	3.8	4.9	0	0	0.9	0.2
<i>pn + + + f</i>	0.9	1.1	1.2	0.7	0	0.8	1.0	1.5	0.2
<i>+ v m wy +</i>	0	0	1.0	0.2	0	0	0	0	3.1
<i>pn + + + wy f</i>	0.1	0	0.1	0.1	0	1.3	0	0	0
<i>+ v m + +</i>	0.1	0	0.4	0.2	0.1	0	0	0	0.2
Total	822	620	1980	453	143	240	103	341	647
Map Position						0.8	3	5	16

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TABLE A-1 - CONTINUED RECOMBINATION DATA OF THE MUTANTS

	<i>pur</i> 1-2	<i>gua</i> 1-1 <sup>ts</sup>	<i>e</i> <i>pur</i> 1-1	<i>yea</i> 1-1	<i>b</i> <i>yea</i> 6-1 <sup>ts</sup>	<i>b</i> <sub>(<i>r</i>)</sub> <i>pur</i> 1-5 <sup>ts</sup>	<i>yea</i> 7-1 <sup>sd</sup>	<i>r</i> <i>pur</i> 1-15	<i>r</i> <i>pur</i> 1-12
<i>pn v m wy f</i>	55.5	59.6	66.1	66.8	56.6	62.3	71.7	69.3	54.0
<i>+ + + + +</i>	3.0	0	0.3	0.2	6.3	0	0	0	0
<i>pn + + + +</i>	2.0	0.2	0.9	0	0.6	0	0	0	0
<i>+ v m wy f</i>	14.1	16.1	10.4	13.9	9.3	18.4	10.6	13.3	19.9
<i>pn v + + +</i>	1.7	2.4	1.6	0	0.6	0	0	0	0
<i>+ + m wy f</i>	0	0	0	1.9	3.0	3.5	0.4	3.0	2.5
<i>pn v m + +</i>	3.7	4.4	3.8	3.5	0	0	0	0	0
<i>+ + + wy f</i>	0.7	0	0	0.3	6.6	4.4	2.9	4.8	3.1
<i>pn v m wy +</i>	13.5	14.3	15.2	12.0	11.4	1.8	3.7	2.2	4.3
<i>+ + + + + f</i>	1.3	0	0	0	4.5	8.8	8.2	6.3	11.8
<i>pn + + + + f</i>	0	0	0	0	0.6	0	1.6	1.1	2.5
<i>+ v m wy +</i>	3.7	1.6	1.6	1.1	0.6	0.8	0	0	0
<i>pn + + + wy f</i>	0	0	0	0	0	0	0.4	0	0.6
<i>+ v m + +</i>	0.7	1.4	0	0.3	0	0	0	0	1.2
Total	297	498	316	1158	334	114	244	271	161
Map Position	30	33	31	366	45	53	52	53	53

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TABLE A-1 - CONTINUED RECOMBINATION DATA OF THE MUTANTS

	( <i>r</i> )pyr 1-4	<i>r</i> pyr 1-8	<i>r</i> pyr 1-13	<i>r</i> pyr 1-14	( <i>r</i> )pyr 1-16	<i>r</i> pyr 1-18	( <i>r</i> )pyr 1-1	<i>r</i> pyr 1-17
<i>pn v m wy f</i>	67.2	64.1	69.2	71.3	66.2	64.1	69.8	67.9
<i>+ + + + +</i>	0	0	0	0	0.5	0	0.3	0
<i>pn + + + + +</i>	0	0	0	0	0	0	0.2	0
<i>+ v m wy f</i>	12.7	17.9	11.9	9.4	12.6	17.9	11.9	14.7
<i>pn v + + + +</i>	0	0	0	0	0	0	0	0
<i>+ + m wy f</i>	2.9	0	1.4	2.1	2.4	0	1.8	2.7
<i>pn v m + + +</i>	0	0	0	0	0	0	0	0
<i>+ + + wy f</i>	4.1	3.4	4.6	7.7	4.3	3.4	3.0	2.2
<i>pn v m wy +</i>	1.0	0.9	1.4	1.2	1.9	0.9	0.8	0.4
<i>+ + + + f</i>	11.1	12.0	10.0	8.2	11.1	12.0	11.1	10.3
<i>pn + + + + f</i>	0.3	0.9	1.4	0	0	0.9	0	0.4
<i>+ v m wy +</i>	0.6	0.9	0.2	0	1.0	0.9	0.8	0.8
<i>pn + + + wy f</i>	0	0	0	0	0	0	0	0
<i>+ v m + + +</i>	0	0	0	0	0	0	0.3	0.4
Total	314	117	438	244	207	117	606	224
Map Position	55	55	55	55	55	55	56	56

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TABLE A-1 - CONTINUED RECOMBINATION DATA OF THE MUTANTS

	$r$ pyr 1-11	$b_{(r)}$ pyr 1-7 <sup>ts</sup>	$b_{(r)}$ pyr 1-6 <sup>ts</sup>	$(r)$ pyr 1-20	$r$ pyr 1-10	$(r)$ pyr 1-3 <sup>ts</sup>	$(r)$ pyr 1-2
<i>pn v m wy f</i>	67.7	50.4	68.8	60.8	70.1	68.3	64.4
<i>+ + + + +</i>	0	4.4	0.6	0	0	0	1.3
<i>pn + + + +</i>	0	1.2	0	0	0	0.2	0
<i>+ v m wy f</i>	15.5	18.3	13.8	20.5	11.2	11.5	15.5
<i>pn v + + +</i>	0	0	0	0	0	0	0
<i>+ + + + +</i>	1.8	2.4	2.3	2.3	3.9	1.8	1.8
<i>pn v m + +</i>	0	0	0	0	0	0	0
<i>+ + + wy f</i>	2.5	5.2	2.9	4.6	2.7	4.2	2.8
<i>pn v m wy +</i>	2.8	3.2	2.9	2.3	1.9	1.8	1.5
<i>+ + + + + f</i>	9.1	12.7	6.9	7.6	8.9	10.5	10.5
<i>pn + + + + f</i>	0	0.4	1.7	1.8	0.8	0.9	1.5
<i>+ v m wy +</i>	0.3	2.0	0	0	0.4	0.3	0.5
<i>pn + + + wy f</i>	0.3	0	0	0	0	0.1	0.3
<i>+ v m + + +</i>	0	0	0	0	0	0.4	0
Total	394	252	173	171	258	707	399
Map Position	53	53	53	54	54	54	55

continued on next page



TABLE A-1 - CONTINUED RECOMBINATION DATA OF THE MUTANTS

	<i>r</i> <sub>pyr</sub> 1-19	<i>ade</i> 1-1 <sup>sd</sup>	<i>yea</i> 5-1
<i>pn v m wy f</i>	60.5	63.0	57.3
<i>+ + + + +</i>	0	1.5	7.8
<i>pn + + + +</i>	0	0.6	2.0
<i>+ v m wy f</i>	20.0	14.7	12.9
<i>pn v + + +</i>	0	0	0
<i>+ + m wy f</i>	3.0	1.8	1.2
<i>pn v m + +</i>	0	0	0
<i>+ + + wy f</i>	1.3	5.8	5.1
<i>pn v m wy +</i>	0.7	0	0
<i>+ + + + f</i>	11.4	10.8	12.8
<i>pn + + + f</i>	0	1.5	0.8
<i>+ v m wy +</i>	3.0	0	0
<i>pn + + wy f</i>	0	0.2	0.4
<i>+ v m + +</i>	0	0	0
Total	299	544	255
Map Position	56	57	66

(footnotes on the following page)



TABLE A-1 - CONTINUED RECOMBINATION DATA OF THE MUTANTS

- a Progeny were scored over the first two days of emergence only.
- b Larvae were cultured at 25°C, embryos and pupae at 29°C.
- c Cultures maintained at 29°C for the full life cycle.
- d Progeny were scored on the first day of emergence only.
- e Flies with small body size were considered escapers and were not counted.

Male progeny of the cross *pn v m wy f* / mutant females X *pn v m wy f* males were scored as shown. All values are expressed as percentage of the total. Culture temperature was 25°C, unless otherwise indicated. Map positions were calculated as shown in Table A-2.



TABLE A-2

CALCULATION OF THE MAP POSITIONS<sup>#</sup>

With respect to recombinants in the interval containing the mutation, "a" represents the number of males carrying the mutant allele of the proximal marker of the interval, and "b" represents the number of males carrying the wild type allele of the proximal marker.

$\ell$  = the frequency of escapers as determined by the number of + + + + + males relative to *pn v m wy f* males.

R = theoretical map distance of the interval containing the mutation

M = Map position of the distal marker of the interval containing the mutation

M' = Map position of the mutation. Map position (M') was determined by the following formula: 
$$M' = \frac{(a - \ell a) R}{(a + b) - \ell (a + b)} + M$$

MUTANT DESIGNATION	MUTANT INTERVAL	a	b	$\ell$	M'
<i>yea</i> 3-1	<i>pn - v</i>	0	57	0	0.8
<i>yea</i> 4-1 <sup>sd</sup>	<i>pn - v</i>	1	15	"	3
✓ <i>yea</i> 4-2 <sup>sd</sup>	<i>pn - v</i>	7	60	.01	5
<i>yea</i> 2-1 <sup>ts</sup>	<i>pn - v</i>	44	50	.1	16
<i>pur</i> 1-2	<i>pn - v</i>	55	6	.06	30
<i>gua</i> 1-1 <sup>ts</sup>	<i>pn - v</i>	38	3	0	31
<i>pur</i> 1-1	<i>pn - v</i>	96	1	0	33
<i>yea</i> 1-1	<i>m - wy</i>	4	44	0	36.6
<i>yea</i> 6-1 <sup>ts</sup>	<i>wy - f</i>	15	49	.08	45
( <i>r</i> ) <i>pyr</i> 1-5 <sup>ts</sup>	<i>wy - f</i>	25	12	0	52
<i>yea</i> 7-1 <sup>ts</sup>	<i>wy - f</i>	10	4	"	53
<i>rpyr</i> 1-15	<i>wy - f</i>	20	6	"	53
<i>rpyr</i> 1-12	<i>wy - f</i>	23	9	"	53
<i>rpyr</i> 1-11	<i>wy - f</i>	37	12	"	53
( <i>r</i> ) <i>pyr</i> 1-7 <sup>ts</sup>	<i>wy - f</i>	37	11	"	53
( <i>r</i> ) <i>pyr</i> 1-6 <sup>ts</sup>	<i>wy - f</i>	16	6	"	53
( <i>r</i> ) <i>pyr</i> 1-20	<i>wy - f</i>	17	4	"	54
<i>rpyr</i> 1-10	<i>wy - f</i>	25	6	"	54

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TABLE A-2 - CONTINUED      CALCULATION OF THE MAP POSITIONS<sup>#</sup>

MUTANT DESIGNATION	MUTANT INTERVAL	a	b	ℓ	M'
( <i>r</i> ) <i>pyr</i> 1-3 <sup>ts</sup>	wy - f	81	15	0	54
( <i>r</i> ) <i>pyr</i> 1-2	wy - f	49	8	.02	55
( <i>r</i> ) <i>pyr</i> 1-4	wy - f	36	5	0	55
<i>r</i> <i>pyr</i> 1-8	wy - f	28	3	0	55
<i>r</i> <i>pyr</i> 1-3	wy - f	50	7	0	55
<i>r</i> <i>pyr</i> 1-14	wy - f	20	3	0	55
( <i>r</i> ) <i>pyr</i> 1-16	wy - f	25	4	0	55
<i>r</i> <i>pyr</i> 1-18	wy - f	16	2	0	55
( <i>r</i> ) <i>pyr</i> 1-1	wy - f	72	5	.01	56
<i>r</i> <i>pyr</i> 1-17	wy - f	25	2	0	56
<i>r</i> <i>pyr</i> 1-19	wy - f	43	2	0	56

\* Escapers were identified by small body size (number not recorded). Data was taken from crosses of the type described in Table A-1.

# Data has been taken from Table A-1.

✓ Control Data (see Table A-1) showed a significant difference between the *pn* + + + + and + *v* *m* *wy* *f* classes. The values shown have been corrected for this difference.



TABLE A-3      MAP POSITION OF MUTANTS IN THE *FORKED* -  
CENTROMERE INTERVAL

For mutations located between forked and the centromere the *pn v m wy f* stock is not reliable. Recombinants of the "a" type (see Table A-2) carry *pn v m wy f* and cannot be distinguished from non-recombinants. For mutants of this type *y sc v f y<sup>+</sup>* (stock 5, Table 1) was used. Females heterozygous for *y sc v f y<sup>+</sup>* and the mutant chromosome were crossed to *y sc v f y<sup>+</sup>* males. Male progeny of the "a" type (*y sc v f*) and "b" type (*+ + + + y<sup>+</sup>*) were counted. The resulting map position is only reliable if the mutation is "non-leaky", since *+ + + +* flies cannot be distinguished from *+ + + + y<sup>+</sup>*. Both mutations are "non-leaky" (in  $\overline{XXY}$  experiments) under the conditions used in these experiments. Map positions were determined by the formula in Table A-2.

MUTANT DESIGNATION	a	b	M'
* <i>ade</i> 1-1 <sup>sd</sup>	2	33	57
<i>yea</i> 5-1	74	10	66

\* scored on the first day of emergence only



TABLE A-4

## PREVIOUS DESIGNATIONS OF MUTANTS

Throughout the course of these experiments a system of nomenclature different from that presented in this thesis has been used. In order to alleviate any possible confusion from this change, the old and new designations are shown below:

MUTANT	PREVIOUS DESIGNATION	MUTANT	PREVIOUS DESIGNATION
<i>yea</i> 1-1	FNC 8	( <i>r</i> ) <i>pyr</i> 1-5 <sup>ts</sup>	FNC 38
<i>yea</i> 2-1 <sup>ts</sup>	FNC 19	( <i>r</i> ) <i>pyr</i> 1-6 <sup>ts</sup>	FNC 39
<i>yea</i> 3-1	FNC 35	( <i>r</i> ) <i>pyr</i> 1-7 <sup>ts</sup>	FNC 40
<i>yea</i> 4-1 <sup>sd</sup>	FNC 46	<i>rpyr</i> 1-8	FNC 41
<i>yea</i> 4-2 <sup>ts</sup>	FNC 55	<i>rpyr</i> 1-9	FNC 42
<i>yea</i> 5-1	FNC 49	<i>rpyr</i> 1-10	FNC 43
<i>yea</i> 6-1 <sup>ts</sup>	FNC 53	<i>rpyr</i> 1-11	FNC 44
<i>yea</i> 7-1 <sup>sd</sup>	FNC 57	<i>rpyr</i> 1-12	FNC 47
<i>gua</i> 1-1 <sup>ts</sup>	FNC 7	<i>rpyr</i> 1-13	FNC 48
<i>pur</i> 1-1	FNC 34	<i>rpyr</i> 1-14	FNC 50
<i>pur</i> 1-2	FNC 45	<i>rpyr</i> 1-15	FNC 51
<i>ade</i> 1-1 <sup>sd</sup>	FNC 5	( <i>r</i> ) <i>pyr</i> 1-6	FNC 52
( <i>r</i> ) <i>pyr</i> 1-1	FNC 22	<i>rpyr</i> 1-17	FNC 54
( <i>r</i> ) <i>pyr</i> 1-2	FNC 33	<i>rpyr</i> 1-18	FNC 58
( <i>r</i> ) <i>pyr</i> 1-3 <sup>ts</sup>	FNC 36	<i>rpyr</i> 1-19	FNC 59
( <i>r</i> ) <i>pyr</i> 1-4	FNC 37	( <i>r</i> ) <i>pyr</i> 1-20	FNC 60





















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